# EVALUATION OF T CELLS & LANGERHANS CELLS IN LICHEN PLANUS & LICHENOID MUCOSITIS - IMMUNOHISTOCHEMICAL STUDY

***Dissertation submitted to***

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***In partial fulfillment for the Degree of***

MASTER OF DENTAL SURGERY

BRANCH IV

ORAL AND MAXILLOFACIAL PATHOLOGY

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# CERTIFICATE

This is to certify that this dissertation titled "**EVALUATION OF T CELL & LANGERHANS CELL IN LICHEN PLANUS & LICHENOID MUCOSITIS - IMMUNOHISTOCHEMICAL STUDY** " is a bonafide record of work done under our guidance during the study period between 2003-2006.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, in partial fulfillment for the Degree of **MASTER OF DENTAL SURGERY - ORAL AND MAXILLOFACIAL PATHOLOGY, BRANCH IV.** It has not been submitted (partial or full) for the award of any other degree or diploma.

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# CONTENTS

# *Introduction*

Lichen planus (LP) is a chronic mucocutaneous disease affecting skin and mucous membrane. It causes bilateral white striations, papules or plaques on the buccal mucosa, tongue, and gingiva. It results due to a cell mediated immunological response to an antigenic change in the skin or mucosa[35].

Lesions that appear clinically identical to LP, but caused by drugs are called Lichenoid drug eruption (LDE). LDE involving the oral mucous membrane is also termed as Lichenoid mucositis (LM). It occurs after the administration of systemic drugs such as NSAID, Sulphonyl ureas, Antimalarials, Betablockers, ACE inhibitors, Diuretics, Gold salts and Heavy metals[15]. Clinical and histopathological features of LM & LP are similar in many respects.

In oral LP, it has been hypothesized that the initiation of the disease process is triggered by the permeation of unknown antigens from the oral cavity into the oral epithelium[17]. The passage of antigens through the epithelium is by the entrapment of these antigens by Langerhans cells (LC). LC are immunocompetent dendritic cells that form a network within the epithelium. They process antigens for presentation to CD4 helper/inducer T lymphocytes, which in turn will activate CD8 suppressor/cytotoxic T lymphocytes. These CD8 cells are responsible for the basal cell degeneration seen in LP[41].

In LM associated with systemic drug administration, the mode of access of the antigen to the immune system is unlikely to be directly across the epithelium[41]. It probably involves a more remote site of antigen processing and presentation in the epithelium without the involvement of LC. This explains the less LC counts in LM as

compared to LP and this is due to different modes of antigen processing and presentation[41].

Human CD45 is expressed on all cells of hematopoietic origin, except erythrocytes. CD45 is a transmembrane tyrosine phosphatase which can exist in at least nine different isoforms resulting from tissue-specific alternative RNA splicing of exons 4-7 of a single gene coding for the various N-terminal peptide segments[1]. Isoforms of CD45 proteins that are expressed on a restricted group of cell types are designated as CD45R. Most naive human T cells, recognized as CD45RA, express a form of CD45R and contain a segment encoded by an exon designated A. Memory T cells express a different isoform called CD45RO that contains none of the alternatively spliced exons. They are present on most thymocytes, a subpopulation of resting (memory) T cells within both CD4 and CD8 subsets as well as on monocytes and granulocytes[1].

When naive cell encounter antigen, they differentiate into effector lymphocytes that have functions in protective immune responses. Naive-T-cells produce IL-2 which functions as growth & differentiation factor for T-cells[1]. Effector T cells includes cytokine secreting CD4+ helper T cells and CD8+ cytotoxic T cells. Some of the progeny of antigen activated T cells differentiate into memory cells that survive for long periods in a quiescent state. These memory cells are responsible for the rapid and enhanced responses to subsequent exposures to antigen[1].

With this background, we did this study to compare the clinical and histopathological features of LP & LM and to evaluate the number & activity of LC and T cells, by using monoclonal antibody CD1a & CD45RO, to determine the possible different immunopathogenic mechanisms of these lesions.

# *Aims & Objectives*

## HYPOTHESIS

There is an increase in expression of T cells and Langerhans cells in Lichen planus when compared to Lichenoid mucositis

## AIMS AND OBJECTIVES

1. To identify & evaluate LC in LP, LM and normal mucosa using CD1a monoclonal antibody immunohistochemically.
2. To identify & evaluate memory T cells in LP, LM and normal mucosa using CD45RO monoclonal antibody immunohistochemically.

# *Review of Literature*

## LANGERHANS CELL

In 1868 Paul described a dendritically shaped cell population located in squamous epithelia of the epidermis, in the suprabasal layers, by using a gold chloride impregnation technique. For many years Langerhans cells (LC) were regarded as ectodermal cells, artifacts, melanocytes or neural elements such as schwann cells. These cells represent about 4% of epidermal cells in man[39].

For almost 100 years, these cells remained unattended. With the aid of electron microscope Birbeck *et al* demonstrated in these cells the presence of rod shaped intracytoplasmic granules with transverse periodic striations that are considered today as LC. These granules are probably derived from the plasma membrane and they seem to be involved in receptor mediated endocytosis[39].

LC are members of a family of highly specialized antigen presenting cells (APC) termed as Dentritic cells. The term Dentritic has been used to denote members of this family of cells having the same characteristic morphology and immunologic features. This series of cells include LC possessing Birbecks granules, interdigitating cells within T cells area of lymphoid tissues and so called Vieled cells found in afferent lymph vessels[27].

LC are localized at the interface between the organism and the environment and are important sentinels of the immune system. In aggregates, LC form a continuous network of cells that are equipped to ingest intruding microbes and other environmental components and process the complex antigen into small fragments that can be recognized by T cells[27]. The unique migratory ability of LC allow them to transport antigen from

epidermis to regional lymph nodes, where they can initiate a systemic immune response by presenting cell surface bound processed antigen to resting T lymphocytes[27].

## ORIGIN

Since 1868, because of their staining behavior, their dendritic morphology and an apparent continuity with their nerve fibers of the dermis, LC were first thought to represent intraepidermal nerve cells[51].

In late 1940 it was known as LC were aged or worn out melanocytes that had lost their ability to produce pigments, left their basal localization and were in the process of being exfoliated[18].

In 1961, detailed description of the ultra morphology LC with demonstration of Birbeck granule has become the hallmark of LC[39].

In 1979, Katz and Frelinger[27] *et al* introduced the concept that LC are derived from a mobile pool of bone marrow precursors. Although LC are clearly myeloid cells, immediate precursors of LC have not been identified. There may be a small population of LC that can proliferate *insitu*, but it is generally agreed that the bulk of LC are postmitotic[27]. Thus LC must derive from blood borne committed precursor or from less differentiated precursors that assumes the characteristic of LC locally[27]. In 1996, Winning *et al* suggested that LC are derived from monocyte lineage of bone marrow cells[68].

LC was thought to be of mesenchymal origin after Birbeck granules were identified in Histiocytosis X cells and this introduced the concept of LC as immune cell. Close apposition of lymphocytes and LC in the epidermis, accumulation of LC in

lymphatics, increased LC in dermis after antigen challenge led to the concept that LC might transport antigen acquired in the epidermis to regional lymph nodes[27].

The demonstration of Fc and complement receptors, as well as MHC on the surface of LC finally consolidated their role as a cell of immune system. LC are members of a family of highly specialized antigen presenting cells (APC) termed as dendritic cell system[27].

Even in the absence of obvious stimulation there seems to be a slow, but continuous turn over of LC. Local activation of LC by contact allergen or irritants lead to a dramatic increase in the number of LC that leave the epidermis and migrate to regional lymph nodes via the afferent lymphatics. During this phase of their life history, LC undergo dramatic functional and phenotypic changes that enable them to act as potent APC activating unprimed T cells in the paracortical areas of regional lymph nodes[27].

Not until the late 1980 it was conclusively demonstrated that LC could take up antigen in epidermis, migrate to regional lymph nodes and present imported antigen to naive T lymphocytes in a MHC restricted manner. Freshly obtained LC and LC *insitu* are effective in taking up and processing protein, microbial and particulate antigen but present antigen inefficiently. Only after activation (eg by contact allergens, microbial products or proinflammatory cytokines) do LC leave the epidermal compartment and migrate via afferent lymphatics to regional lymph nodes[27].

During this process, LC upregulates surface expression of MHC and co stimulatory molecules and thus acquire the functional characteristics of professional APCs when they reach the T cell area of the draining lymph node. This transition in LC phenotype and functional activity is called LC maturation[27].

## FUNCTION

LC are specialized cells with immune functions resembling those of other dendrite cells and of macrophages. In fact, LC have been shown to be able to migrate and to handle antigen in a manner similar to that of the antigen presenting cells such as macrophages, B cells and other dendritic cells. LC expresses MHC class II antigens at their surface, and through these molecules it can present the antigen to T cells[39] .

It is now well established that T lymphocytes do not recognize the antigen by itself. This needs first to be taken up by APC, degraded (processed) by proteolytic digestion, and re-expressed (presented) at their surface in the context of the MHC molecules. T cells then recognize the antigen under the form of an epitope associated with class I & class II molecules[39]. The recognition of an epitope in the context of MHC class II molecules (DR, DP, DQ of the HLA in humans) is essentially done by T helper (CD4) cells, whereas T cytotoxic (CD8) cells mainly recognize the epitope when it is associated with class I molecules (HLA-A, HLA-B, HLA-C). This is commonly defined as MHC restriction of T cell activation[39].

Other non-specific factors like LFA-1, ICAM-1, LFA-3 as well as certain cytokines such as IL-1, IL-6 can act on T cell activation depending on the activation and maturation of the T cell[39].

It is now established that epidermal cells can induce antigen driven proliferation of sensitized T cells and that this property is essentially linked to the presence of class II bearing LC. Using ‘ purified’ epidermal LC, several authors have shown that LC can present antigen to both helper and cytotoxic T cells specific for protein antigens, haptens

and alloantigens and that the removal of LC from the epidermal cell population eliminates this stimulatory effect[39].

It is reasonable to postulate that resident LC take up the antigens, thus specifically stimulating resting T helper and cytotoxic cells either locally or, after migration, in the draining lymph nodes. Furthermore, the similarity existing between resident LC and certain macrophage/monocyte like cell suggests that LC could possess clearance and effector functions, as well as the property of secreting active mediators IL-1. All this would suggest a key role for LC in the induction of protective immune responses against a wide variety of antigenic stimuli and also of producing immunopathological reactions taking place at cutaneous and /or mucosal level[39].

Considering the fact that skin and mucosae are continuously exposed to a variety of injurious agents, often having antigenic features, LC may well represent a 'first line' of sensitization of the immune system, leading to clearance of the antigen or to a pathological phenomena. It is reasonable to see LC as participating in the pathogenesis of some cutaneous and mucosal pathological entities[39].

**IDENTIFICATION:**

*Classical histology:*

LC appears as high-level clear cell in light microscopy sections stained with H & E. Certain particular methods also can detect this cell. Technique employed were metal impregnation staining such as gold, osmium iodide or zinc osmium iodide[39].

*Histochemistry:*

The demonstration of a membrane bound, formalin resistant, and sulfhydryl dependant adenosine triphosphatase (ATP-ase) is an excellent method for identification

of human LC. These cells are predominantly found at a suprabasal level and rarely in the basal layer. Although the dendrites may give the impression of forming an interconnected network, unequivocal contact between LC remains to be demonstrated[39].

*Electron microscopy:*

The Birbeck granules seem to be specific marker of LC. They possess tennis racket morphology with transverse striations. Their function was presumed to be receptor-mediated endocytosis. It has been recently suggested that at least a part of the cytoplasmic Birbeck granules are not isolated cytoplasmic organelles but they result from a fragmentation of the tubular structure called 'the continuous endosomal reticulum' [39].

Two types of LC have been described:

Type I – Highly dendritic with an electronlucent cytoplasm, numerous granules and is usually found in the suprabasal layer.

Type II - Shows fewer dendrites, a more electron dense cytoplasm, fewer Birbeck granules and is located in the basal layer[39].

Six electron microscopic criteria of specificity for LC have been used:

1. Indented or lobulated nucleus
2. Birbeck granules
3. Absence of tonofilaments
4. Well developed golgi apparatus with a clear cytoplasm
5. Absence of desmosomes
6. Absence of melanosomes & premelanosomes.

LC has a prominent network of microfilaments as well as a system of microtubules. Both these features are evidences of a capacity to migrate[39].

*Immunolabeling:*

A large number of surface and/ or cytoplasmic antigens have been localized on LC by means of antibodies used in either immunofluorescence or immunoperoxidase techniques. Though many or even all of them are important for LC functions, only those antigen/antibody reactions that are displayed by all LC but not expressed by other cells within the epithelium can be employed for LC identification[39].

One of the most prominent features of LC is the high level of expression of MHC class II antigens. In addition to the MHC class II antigens, MHC class I antigens have been shown on LC as well as the leukocyte common antigen (CD45). The latter antigen is a marker for cells of haemopoietic origin and stresses the bone marrow origin of LC[39].

Molecules within the group of integrins (adhesion molecules) involved in cell-to-cell interactions have also been demonstrated on LC. These molecules include the lymphocyte function associated antigen LFA-1 (CD11a) and the very late activation antigen VLA (CDw29). CD4, originally considered to be restricted to the helper lymphocyte subpopulation, has also been identified on LC. This antigen plays an important role in the activation of T cells, together with the T cell receptor and MHC class II antigens[39].

Among the markers used, anti CD1a (T6) immunolabeling is considered to be the most reliable to identify the human LC in the epithelium[39].

## T LYMPHOCYTE

Cell mediated immunity is mediated by T lymphocytes. T-cells occur either as helper T-cells, which assist B-cells in the production of antibody, or as cytotoxic T-cells, which stimulate the microbiocidal and cytotoxic activity of other immune cells including macrophages. Lymphocyte-mediated recognition of antigen shows specificity, as well as memory[1].

T lymphocytes recognize the antigens through *antigen receptors*, membrane molecules distinct from but structurally related to antibodies. Antigens are captured from their site of entry by dentritic cells and concentrated in lymph nodes, where they activate naïve lymphocytes that migrate to the nodes through blood vessels. Effector and memory T cells develop in the nodes and enter the circulation from which they migrate to peripheral tissues[1].

T cells develop diversity for reacting against different specific antigens. The activated T-helper cells then produce factors, which stimulate B-cells to undergo differentiation into plasma cells and eventually produce antibodies. Activated T-helper cells also produce factors, which stimulate cytotoxic cells and phagocytes, arming them for microbiocidal or cytotoxic activity[1].

## CHARACTERISTICS OF NAÏVE, EFFECTOR & MEMORY LYMPHOCYTES

Naive T cells are unprimed lymphocytes that have been stimulated by antigen to become immune competent lymphocytes. When they encounter antigen, they differentiate into effector lymphocytes that have a function in protective immune responses. Naive-T-cells produce IL-2 which functions as growth & differentiation factor for T-cells. Effector T cells includes cytokine- secreting CD4+ helper T cells

and CD8+ Cytotoxic T cells. Some of the progeny of antigen activated T cells differentiate into memory cells that survive for long periods in a quiescent state. These memory cells are responsible for the rapid and enhanced responses to subsequent exposures to antigen[1].

## ROLE OF CD45 IN T CELL ACTIVATION

CD45 a cell surface glycoprotein with a cytoplasmic tyrosine phosphatase domain is believed to play a role in T cell activation. Various forms of CD45 are expressed on immature and mature leukocytes, including T and B cells, thymocytes, mononuclear phagocytes and polymorphonuclear leukocytes. The CD45 family consists of multiple members that are all products of a single complex gene. This gene contains 34 exons, and the primary RNA transcripts of three of the exons (called A, B and C) are alternatively spliced to generate up to eight different messenger RNAs and eight different protein products[1].

The predicted aminoacid sequences of the protein products include external domains of varying lengths, a transmembrane region and a 705-aminoacid cytoplasmic domain that is one of the largest identified among membrane proteins. Isoforms of CD45 proteins that are expressed on a restricted group of cell types are designated as CD45R. Most naïve human T cells express a form of CD45R that is called CD45RA, whereas memory T cells express a different isoforms called CD45RO[1].

## LICHEN PLANUS

Lichen planus is a unique, common inflammatory disorder that affects the skin, mucous membrane, nails and hair. In *Greek* 'Leichen' means 'tree moss' and in *Latin* 'planus' means 'flat'. Because Leichens are primitive organisms of symbiotic algae and fungi, it can be assumed that the clinical appearance of lesions LP represent reminiscent of liechens growing on rocks. The term suggests it as a flat fungal infection but current evidence proposes it as a mucocutaneous disorder mediated by numerous complex immunologic events[21].

## HISTORY[21]

- Erasmus Wilson was the first person who described the condition leichen (lichen) planus in1869.
- Kaposi first described a distinctive clinical variant of the disease with blisters, *lichen rubber pemphigoides,* in 1892.
- Wickham described the characteristic appearance of whitish striae and punctuations that develop atop the flat surface papules in 1895.
- Dubreuill first described the histopathological features of OLP that are characteristic and similar to those of cutaneous LP in 1906
- Darier elaborated the histological findings in1909.

## ETIOLOGY

LP is multifactorial in origin with an immunopathogenesis involving T cells in particular. The etiopathogenesis appear to be complex, with interaction between genetic, environmental, and lifestyle factors and new associations such as with liver disease.

**i) <u>AUTOIMMUNITY</u>**

**Sugerman PB, Savage NW, Walsh LJ *et al* (1993)**[60] proposed that in oral lichen planus (OLP) diverse exogenous agents such as drugs, trauma & infection stimulate the expression of a common self-molecule by oral mucosal keratinocytes. An autoimmune reaction by cytotoxic T lymphocytes to these activated keratinocytes may result in the tissue destruction, which is characteristic of OLP.

**Bramanti TE, Dekker NP, Nur FL *et al* (1995)**[6] studied Heat shock proteins (HSP) as the antigen stimulus in autoimmune diseases. They concluded that although the expression of HSP was altered in LP, the difference demonstrated was slight and were therefore inconclusive. The HSP contribute to the persistence or chronicity of the disease, or they could have simply reflected cellular injury.

**Chaiyarit P, Kafrawy AH, Miles DA *et al* (1999)**[10] hypothesized that in a genetically predisposed individual; a hapten, a conventional antigen or a super antigen of oral microbial origin could induce a cell-mediated immune response with subepithelial infiltration of T cells. Generation of cytokine by these cells may upregulate the expression of Heat shock proteins HSP 60 in the adjacent basal keratinocytes. If the individual is predisposed to react to HSP 60 by virtue of possession of certain HLA antigen then a second immune reaction follows with development of cytotoxic T lymphocyte that target basal keratinocyte causing autoimmune reaction and destruction of basal cells. The author concludes suggesting that HSP 60 plays a role in the pathogenesis of OLP.

**ii) INFECTION**

**Sand LP, Jalouli J, Larsson PA *et al* (2002)**[53] studied that Epstein Barr virus (EBV) is present in oral diseases such as oral squamous cell carcinoma (OSCC) and OLP. Of the 23 OLP patients, 26.1% were EBV positive.

**Chainani-Wu N, Lozada-Nur F, Terrault N (2004)**[8] reviewed an association between OLP and HCV infection by a search of the computerized database MEDLINE (1966-June 2003). Biases-including selection bias and investigation bias in the studies published-make it difficult to draw firm conclusions.

**Campisi G, Giovannelli L, Arico P *et al* (2004)**[7] evaluated the prevalence of human papillomavirus (HPV) infection in 71 OLP in comparison with that in healthy oral mucosa. An increased risk of HPV infection was found in OLP; however, no specific clinical variant of OLP was noted to be associated with HPV infection.

**Cunha KS, Manso AC, Cardoso AS *et al* (2005)**[14] studied 134 patients with HCV infection. The prevalence of OLP was 1.5% in patients with HCV infection and 1.1% in the control group. There was no statistically significant difference between the two groups (p- 0.630) in Brazilian patients.

**iii) STRESS**

**McCartan BE (1995)**[40] studied 50 patients with OLP for current anxiety and depression and for related personality factors. There were no statistically significant associations between erosive oral LP and either anxiety or depression.

**Garcia-Pola M.J, Huertaa G, Cereros R *et al* (2001)**[22] suggested that anxiety and depression constitute risk factors that could influence the development of OLP.

**Koray M, Dugler O, Horasanli S *et al* (2003)**[33] studied the evaluation of anxiety and salivary cortisol levels in 40 patients with OLP and found that salivary cortisol and anxiety levels in OLP group were significantly higher than in the control group.

**Chaudhary S (2004)**[12] studied psychosomatic factors and their association in LP. Significantly higher stress, anxiety and depression levels were found in the OLP than the general population. These suggest that psychological stresses play an important role in the causation of OLP. It may be further hypothesized that these stresses form a starting point for the initiation of various autoimmune reactions, which have been shown to the pathogenesis of OLP.

**iv) <u>CELL MEDIATED IMMUNITY</u>**

**Zhao ZZ, Sugerman PB, Zhou XJ *et al* (2001)**[72] studied that OLP lesional T cells produce & secrete RANTES, which triggers human mast cell degranulation. Degranulating mast cells release TNF alpha which upregulates OLP lesional T cell RANTES secretion. Such a cyclical mechanism may underlie disease chronicity.

**Zhao ZZ, Savage NW, Sugerman PB *et al* (2002)**[73] showed the interaction between mast cell & T cells of immune regulation contributing to cell mediated inflammatory response of OLP.

**Zhao ZZ, Sugerman PB, Walsh LJ *et al* (2002)**[71] hypothesized that RANTES & CCR1 may play important roles in mast cell trafficking & related events in OLP.

**Sugerman PB, Savage NW, Walsh LJ *et al* (2002)**[59] reviewed both antigen specific & non antigen specific mechanisms may be involved in the pathogenesis of OLP. Antigen specific mechanism includes antigen presentation by basal keratinocytes and antigen specific keratinocytes killing by CD8 cytotoxic cells. Antigen non-specific

mechanism includes mast cell degranulation and MMP activation. These mechanisms may combine to cause T cell accumulation in the superficial lamina propria, basement membrane distribution, intra epithelial T cell migration and keratinocyte apoptosis in OLP.

**Khan A, Farah CS, Savage NW *et al* (2003)[31]** reported cell mediated immune response in OLP may be regulated by cytokines and their receptors. They suggested that the development of a T helper$_1$ immune response might promote CD8 cytotoxic T cell activity in OLP.

**Walsh LJ (2003)[64]** suggested mast cell proteases might contribute to alterations in the basement membranes in inflammation in the oral cavity, such as the distributions that allow cytotoxic lymphocytes to enter the epithelium in OLP.

## CLINICAL FEATURES

Lichen planus often affects the oral mucosa and often may occur without skin lesions. About half of the patients with skin lesions have oral lesions, whereas about 25% present with oral lesions alone. The prevalence of LP in the general population is estimated as 0.9% - 1.2%, whereas the prevalence of OLP has been reported between 0.1% - 2.2%. OLP is the disease of adulthood occurring at ages ranging from 30 – 70 yrs and about 60 – 65% of the patients being females[28].

OLP may be found in any locations but favored sites are the buccal mucosa, the tongue, and the gingiva, whereas palatal lesions are uncommon. The lesions are always bilateral[28]. OLP is classified according to its clinical features as reticular, papular, plaque, bullous, atrophic, erosive, and ulcerative. Reticular LP is the most common form and predominantly affects buccal mucosa, appearing as a network of white or grey threads

(Wickham's striae) interspersed with papules or rings *(Fig 1).* Vesicle and bulla formation has been reported but this is not a common findings. Atrophic lesions may appear with or without erosions. Erosive type is most likely to cause symptoms, which include a spectrum ranging from spontaneous soreness to severe pain, exacerbated by local irritants. OLP may resemble other mucodermatoses such as lupus erythematosus, pemphigoid, leukoplakia, and especially the so-called OLM[28].

## HISTOPATHOLOGY

In 1906, Dubreuill first described the histopathological features. Typical findings include hyperorthokeratinization or hyperparakeratinization with thickening of granular cell layer. The epithelium displays local acanthosis, with inter and intra cellular edema. The saw tooth appearance of the reteridges is commonly found in skin lesions and rarely in OLP[28].

The earliest findings are an increase in the number of epithelial LC. A well-defined band like inflammatory cell infiltrate, consisting mainly of lymphocytes, develops in the subepithelial connective tissue *(Fig 3).* This is followed by liquefaction degeneration of basal cell layer and the appearance of a thin band of eosinophilic material immediately underlying the basement membrane[28].

Colloid bodies (also called as cytoid, hyaline or civatte bodies) may be seen lying either in lower layer of epithelium or within the upper layer of the connective tissue[28]. The civatte bodies are isolated epithelial cells, shrunken with eosinophilic cytoplasm and one or multiple pyknotic nuclear fragments. They represent apoptotic keratinocytes and other necrotic epithelial components that are transported to the connective tissue for phagocytosis[15].

## LICHENOID MUCOSITIS

Lichen planus like eruptions were first reported in military personnel in World War II who had been prescribed antimalarial drugs. This phenomenon has been termed as Lichenoid drug eruptions (LDE). LDE involving the oral mucous membrane was termed as Lichenoid mucositis (LM) or Oral Lichenoid lesions (OLL). LDE was recognized as a variant of LP till 1929. Many of these cases were biopsied to show appearances identical and similar to idiopathic LP. However, the number of drugs confirmed as precipitating LDE in oral mucous membranes is small, and only a few patients with LP have a history of systemic drug therapy with known lichenoid inducing drugs[38].

**Pinkus H (1973)[21]** defined Lichenoid tissue reaction "as one exhibiting epidermal basal cell damage and the chain of histobiologic events resulting from such damage. It is not essential whether damage to the basal cells is primary or is itself due to preceding events in the dermis. Alterations affect both tissues. This tissue reaction may be called 'lichenoid' because lichen planus is the protype."

## ETIOLOGY

**Penneys NS, Ackerman B, Gottlieb *et al* (1974)[46]** reported Quinacrine and Mepacrine, used as antimalarials during World War II, were seen to cause lichenoid lesions. Apart from these drugs, gold was probably the most common agent recognized as initiating a LM.

**Laeijendecker and Van J (1994)[36]** studied that gold salts can cause a range of mucocutaneous lesions, of which oral lichenoid lesions may be the first.

**Van Dis and Parks (1995)**[62] suggested the drugs most commonly implicated in lichenoid reactions are the Non-steroidal anti-inflammatory drugs (NSAID) and the Angiotensin-converting enzyme (ACE) inhibitors.

**McCartan BE and McCreary CE (1997)**[42] studied that LM occurs after the administration of systemic drugs such as NSAID, Sulphonyl ureas, Antimalarials, Betablockers, ACE inhibitors, Diuretics, Gold salts and Heavy metals.

**Scully and Diz (2001)**[56] suggested that LM also may follow the use of HIV protease inhibitiors, Antihypertensive agents, Antimalarials, Phenothiazines, Sulphonamides, Tetracyclines and Thiazide Diuretics.

**Segura-Egea JJ, Bullon-Fernandez P *et al* (2004)**[57] reported a case of oral lichenoid reaction associated to amalgam restoration. Biopsy showed histological changes compatible with oral lichen planus. Other restorations were performed with composite resins, and no reaction was evidenced in the mucosa.

**Issa Y, Brunton PA, Glenny AM *et al* (2004)**[25] concluded that the replacement of amalgam restoration could result in the resolution or improvement of OLLs. The study population consisted of 1158 patients (27% male and 73% female; age range, 23-79 years). From 16% to 91% of patients had positive patch test results for at least 1 mercury compound. Thus topographic relationship between an OLL and an amalgam restoration is a useful-but not conclusive marker.

**Issa Y, Duxbury AJ, Macfarlane TV *et al* (2005)**[26] investigated a total of 51 patients, who had oral lesions related to their dental restorations of which twenty seven (53%) patients had positive patch test. They concluded that OLL might be elicited by

some dental restorations. Replacing restorations adjacent to these lesions is associated with healing in the majority of cases particularly when lesions are in close contact with restorations.

## CLINICAL FEATURES

LM occur unilaterally with mean age of 57 yrs. LP occur at a younger age group (a decade earlier) than LM. For OLL, the latent period between the beginning of administration of a drug and the appearance of the eruptions is about 1 or 2 weeks, or up to 1 month[23].

The latent period is dependent on the offending drug but other factors may also play a role in determining its duration, that is, the dosage of the drug the patient's individual reaction to the drug, and treatment with other drugs. The latent period may be shortened significantly if the patient has been previously exposed to the offending drug[23].

Lesions of LM can be similar to those of idiopathic LP or have an atypical appearance *(Fig 2).* LM may have eczematous papules and a generalized eczematous skin reaction with marked desquamation. Lesions of LM are considered to be more psoriasiform and larger than lesions of idiopathic LP[23].

Wickham's striae are usually, but not always, absent in lesions of LM. A greater tendency towards residual hyperpigmentation has been noted in LM. Severe alopecia can accompany LM because of follicular involvement. In some patients decreased sweat production can also occur, atrophy of the dermal portion of the sweat duct has been observed[23].

## HISTOPATHOLOGY

The histological features distinguishing LM from LP are a subepithelial infiltrate, containing eosinophils and plasma cells which is more diffuse and which extend more deeply than in LP, perivascular infiltrate, parakeratosis and the presence of colloid bodies *(Fig 4)*[42].

Despite the reported difference between LP & LM, the WHO criteria for LP do not distinguish between the two conditions (WHO collaborating center for oral precancerous lesions, 1978) [42].

There is no specific test for LM. Although resolution and recurrence of LM on withdrawal and re-exposure to the drug is probably diagnostic, the eruptions may persist for long periods following withdrawal of the drug. However, a combination of a history of current use of a known LDE inducing drug, a histological appearance consistent with LM and a detectable presence of circulating basal cell cytoplasmic autoantibody (BCCA) may help in the diagnosis of LM when drug substitution or withdrawal is impractical[42].

Damage to the basal cells is a feature of both LM & LP, and perhaps initial basal cell damage by drug therapy is involved in initiating the production of these autoantibodies called BCCA. These were significantly associated with systemic drug usage, histologic diagnosis of a LM and a unilateral intraoral distribution of the clinical lesion[38].

## ROLE OF LANGERHANS CELLS IN LP & LM

**Bhan AK, Harrist TJ, Murphy GF *et al* (1981)[3]** studied 4 patient of LP using monoclonal antibodies. Intraepidermal and dermal cells with long cytoplasmic extensions stained with any anti-T6 antibody in all cases, was termed as LC or their precursors. T6-positive cells were seen in greater number than in normal control epidermis and dermis. The results indicate that well-developed lesions of LP are characterized by an increased numbers of LC. These observations support the contention that cellular immunity is important in the pathogenesis of this disorder.

**Sloberg K**, **Jonsson R**, **Jontell M *et al* (1984)[58]** demonstrated an increased amount of Ia-like antigens per number of T6-positive LC in LP of oral mucosa compared to healthy conditions. This increase in expression of LC in LP is a property of LC to improve heir capacity to detect and present antigen to the subepithelial T lymphocytes. The increased expression of Ia-like antigens on LC and the contemporary finding of Ia-like antigens on the subepithelial T-cells support the opinion that the pathogenesis of OLP is mainly a cell-mediated type of immunological reaction.

**Regezi JA, Stewart JC, Lloyd RV *et al* (1985)[50]** studied 20 patients with clinically and microscopically confirmed lichen planus immunohistochemically using S-100 protein. It was concluded that langerhans cells, macrophages, and keratinocytes play important roles in antigen processing and/or phagocytosis during the natural history of this disease. Although there was an actual influx of additional LC into the epithelium in active state of LP, it could be argued that this may be more apparent than real. Increased metabolic activity and antigenic challenge could account greater production HLA-DR antigen expression of resident LC without real influx of LC.

**Arachchi PA, Crane IJ, Scully C *et al* (1989)[2]** studied the epithelial dendritic cells (EDC) in non-specific keratosis, lichen planus and OSCC. Monoclonal antibodies to the human CD1 thymocyte (OKT6) and HLA-DR antigens were used. Significantly more T6+ and DR+ EDC were present in lichen planus tissues than normal controls, non-specific keratosis and OSCC. Significantly fewer T6+ EDC and significantly more DR+ cells were present in the invasive epithelium of squamous cell carcinomas than the overlying/adjacent epithelium of carcinomas, the non-specific keratosis group and the normal tissues. The results suggest that immunological enhancement occurs in lichen planus and possibly immunological impairment may characterize invasive OSCC.

**Bolewska J and Reibel J (1989)[4]** studied that patients with mucosal lesions confined to areas opposing amalgam restorations (contact lesions) show a high rate of allergic reaction towards mercury. These lesions may, therefore, represent a contact hypersensitivity reaction and have a lichenoid appearance. The authors therefore evaluated the presence of lymphocyte subpopulations, LC and the expression of HLA-DR antigens on mucosal keratinocytes in biopsies of contact lesions (Group 1) and in OLP with (Group 2) and without (Group 3) partial contact with amalgam restorations. T lymphocytes dominated in all three groups and LC counts were similar. HLA-DR positive keratinocytes were found in 18-36% of lesions in all three groups. Thus, the immunologic parameters examined are not of value in discriminating between the types of lesions studied.

**Chou MJ and Daniels TE (1989)[13]** compared LC expressing HLA-DQ, HLA-DR and T6 antigens in biopsies from 12 patients with OLP and 8 healthy volunteers. LC expressing each antigen was observed in all the specimens, but in LP the cells were

located in higher levels of the epithelium than in controls. In LP specimens, there were significantly more LC expressing HLA-DQ and T6 than HLA-DR (P - 0.0001 and 0.02 respectively); no such differences were found in normal mucosa.. They concluded that in LP there is modulation of HLA-DR and HLA-DQ antigen expression by LC, or differences in the number of LC expressing those antigens.

**Farthing PM, Matear P, Cruchley AT (1990)**[20] studied the numbers of CD1, HLADR, HLADP and HLADQ +ve intraepithelial dendritic cells in lesions of OLP and normal oral mucosa using a pannel of antibodies. In LP, the cells appeared more dendritic and equal numbers of CD1, HLADR, HLADP and HLADQ positive cells were found, with significantly more than in normal mucosa. These results show that although there is no change in the total number of LC in LP, there is an increase in Class II MHC antigen expression. This suggests that in LP, LC are immunologically active and play a role in lesion development.

**Farthing PM**, **Matear P**, **Cruchley AT** (**1992)**[19] showed in LP, an increase both in the number of LC and the numbers expressing CD4 were found in areas of keratinocyte HLADR expression and compared it with HLADR negative areas and with normal oral mucosa. There was no difference in the numbers of LC or their expression of CD4 between HLADR-negative areas in LP and normal oral mucosa. These results show that the distribution of LC is related to keratinocyte expression of HLADR and suggest that LC entry may be enhanced in these areas. While this enhancement is mediated by CD4/HLADR interaction, other molecules are also likely to be important in controlling LC entry into oral mucosa.

**Lombardi T, Hauser C, Jorgensen BE (1993)[39]** described LC as dendritic bone marrow derived cells situated suprabasally in most stratified squamous epithelia, such as the epidermis and the epithelium of oral mucosa, including the gingiva. LC is thought to act as antigen-presenting cells (APC) during induction of immune responses. The exact role of langerhans cells in the oral mucosa is not fully understood although several investigations suggest that these cells are involved in reactions to antigen challenge under both normal and pathological situations.

**Walton LJ**, **Thornhill MH**, **Farthing PM (1994)[67]** examined the expression of intercellular adhesion molecule-1 (ICAM-1, CD54) and vascular cell adhesion molecule-1 (VCAM-1, CD106) in OLP and normal oral mucosa (NOM). Immunoperoxidase staining showed ICAM-1 expression by vascular endothelium in all biopsies of OLP and NOM whereas endothelial VCAM-1 staining was found in 2/7 NOM and 8/9 OLP. Intraepithelial dendritic cells stained for ICAM-1 in 7/9 and VCAM-1 in 4/9 OLP biopsies. Double immunofluorescence showed dual labeling of LC with CD1a and VCAM-1 in a further 5/12 cases of OLP, but there was no such staining in four NOM. They concluded that induction of ICAM-1 and VCAM-1 on LC and macrophages in OLP could cause these cells to be activated and may contribute to the pathogenesis of OLP by presenting antigen to infiltrating lymphocytes.

**Kirby AC, Olsen I, Farthing PM (1995)[32]** studied the expression pattern of lymphocyte function-associated antigen 3 (LFA-3) in the buccal mucosa of OLP patients and compared it with that of healthy controls so as to investigate the possible role of LFA-3 in cell interactions within OLP lesions. LFA 3 is a surface antigen with broad tissue distribution and mediates T lymphocytes binding via CD2 receptor (LFA2).

Interaction between LFA3 and T cell activation enhances the immune response. LFA-3 was expressed on keratinocytes, LC within the epithelium and on endothelial cells in the lamina propria. They have observed that expression of LFA-3 is apparently elevated within OLP lesions. LFA-3 may play an important role in the pathogenesis of OLP.

**Porter SR, Kirby A, Olsen I (1997)[49]** hypothesized the immunological mechanism associated with LP. They suggested that the numbers of LC might be normal or increased. These cells may be more dentritic in LP than in normal mucosa, suggesting an increase in surface antigen expression or elevated dendritic growth. There is a significant increase in HLA-DP and HLA-DQ and perhaps HLA-DR expression is possibly induced by local cytokine production. LC in LP often appear to accumulate in groups of several cells close to the basal cell layer and might be highly involved in the presentation of antigen to infiltrating T lymphocytes.

**McCartan BE and Lamey PJ (1997)[41]** showed that the numbers of LC expressing the common thymocyte antigen (T6/CD1) are similar in OLP and in normal oral epithelium; however, expression of class II major histocompatibility antigens (HLA-DR/Ia) by LC is greater in LP than in normal epithelium, a phenomenon believed to be associated with activation and antigen presentation. Six patients with LDE and LP were studied. An immunoperoxidase technique was used to demonstrate binding of T6 and HLA-DR antibodies to identify dendritic intraepithelial cells as LC and activated LC, respectively. In LDE, the number of HLA-DR + ve LC was significantly lower than the number of T6 + ve LC ($P < 0.05$).

In LP, the initiation of this process appears to be permeation by antigens into the oral epithelium followed by entrapment of these transepithelial antigens by LC. The LC

is immunocompetent dendritic cells that form a plexus within the epithelium & are also found in the dermis or lamina propria. They process antigens to CD4 lymphocytes that in turn may activate CD8 lymphocytes which may be responsible for basal cell damage.[41]

In LM, associated with systemic drug administration, the route of access of the antigen to the immune system is unlikely to be directly across the epithelium but probably involves a more remote site of antigen processing & presentation, which leads to reduction of LC. The results provide evidence for differences in the routes of antigen presentation in LDE and OLP[41]

**McCartan BE and McCreary CE (1997)**[42] proposed that LC play an important role in initiation of inflammatory process in LP. The unidentified antigens are trapped within the epidermis by a plexus of interdigitating LC and subsequently presented by LC to T lymphocytes. Expression of HLA-DR antigens by LC has been considered to be an evidence of activation.

**Walton LJ, Macey MG, Thornhill MH *et al* (1998)**[66] questioned of whether there is selective recruitment and distribution of intra-epithelial leucocytes in lesions of OLP. They demonstrated changes in intra-epithelial T lymphocyte and LC populations compared with normal oral mucosa and suggest that there is a selective recruitment in OLP. In addition, keratinocyte ICAM-1 expression does appear to be associated with accumulation of infiltrating T lymphocytes and LC.

**Laine J, Happonen RP, Konttinen YT *et al* (1999)** [37] studied inflammatory cells in amalgam-associated, oral lichenoid contact lesions (OLL) in 19 patients by immunocytochemistry using monoclonal antibodies. Ten of the patients displayed allergic patch test (PT) reactions to several mercury compounds and nine were negative.

The number of HLA-D/DR-positive dendritic cells and CD1a-positive LC was significantly lower in the PT-negative than PT-positive patients. HLA-D/DR expression on keratinocytes varied from negative to full thickness staining of the epithelium. These patients also showed a good clinical response after amalgam removal. Consequently, OLL may represent a true delayed hypersensitivity reaction with a trans-epithelial route of entrance of the metal haptens released from dental restorative materials.

**Katou F, Ohtani H, Saaristo A *et al* (2000)**[29] investigated the phenotypic characteristics of LC & their relationship with infiltrating lymphocytes. The dermal LC abundantly expressed CD83, a marker of mature dendritic cell. Furthermore these dermal LC were in close contact with CD45RO lymphocytes, suggesting that LC could stimulate neighboring memory CD4 T cells.

**Hasseus B**, **Jontell M**, **Brune M *et al* (2001)**[24] compared the expression of CD1a+, CD80+ and CD86+ cells in the epithelium of OLP and cGVHD lesions, which had the dendritic morphology of LC. Higher frequencies of CD1a+ LC as well as CD25+ cells were observed in the OLP epithelium than in the cGVHD epithelium. The OLP lesions showed higher frequencies of subepithelial cells expressing CD1a, CD86, CD4, CD8 and CD25 than the cGVHD lesions. In conclusion, cGVHD and OLP show marked differences at the cellular level despite similar clinical appearance. Hence, the findings indicate differences in the regulation of the inflammatory response between the two conditions.

**Villarroel DM, Correnti M, Delgado R *et al* (2001)**[63] concluded that the immunological reaction begins with LC activation, which presents an antigen to CD4+ lymphocytes. Those cells through ICAM-1 and LFA-1 promote epithelial destruction. Afterwards, cytokine production, ICAM-1 and VCAM-1 expression can activate CD8+ lymphocytes leading to the chronic form of the disease.

**Dorrego VM, Correnti M, Delgado R *et al* (2002)**[16] suggested that activated epithelia comprise antigen-presenting Langerhans cells, immunocompetent keratinocytes and subepithelial inflammatory infiltrate. The presence of a high density of leucocyte cells may occur for the expression of a variety of adhesion molecules. They studied 18 OLP and 10 normal oral mucosa using IHC staining for CD4, CD8, CD1a, LFA-1, VCAM-1 and ICAM-1. The results showed an increased number of CD4+, CD8+ and CD1a+ cells in OLP. Serial sections showed CD4+ and CD8+ cells also expressed LFA-1. The expression of ICAM-1 and VCAM-1 were significantly higher in OLP. The immunological reaction begins with Langerhans cells activation, which presents an antigen to CD4+ lymphocytes. Those cells through ICAM-1 and LFA-1 promote epithelial destruction. Afterwards, cytokine production, ICAM-1 and VCAM-1 expression can activate CD8+ lymphocytes leading to the chronic form of the disease.

**Santoro A, Majorana A, Roversi L *et al* (2005)**[54] analysed the presence of different dendritic cell (DC) subsets in 16 biopsies from patients with oral lichen planus (OLP) using immunohistochemistry. A significant increase of CD1a+/Langerin+ Langerhans cells, DC-SIGN+ DC and plasmacytoid DCs (PDCs) were found in the epithelium and in the stroma of OLP biopsies compared to normal oral mucosa. A proportion of DCs was mature DC and expressed S100 or CD11c, typically found in the

interdigitating DCs of nodal T-cell areas. Double staining revealed that mature DCs co-expressed CCR7, thus indicating the development of a nodal migratory phenotype upon maturation. Significant recruitment of PDCs producing IFN-alpha was demonstrated by the expression of MxA within the inflammatory infiltrate and close cell-to-cell contacts between PDCs and mature DCs were observed, with a significant correlation between the numbers of these two populations. Moreover, PDCs were also found to contain Granzyme-B, an associated-cytotoxic granule protein, inducing target cell apoptosis. They suggested that PDCs might promote maturation of DCs and amplify the cytotoxicity of lymphoid cells. Finally, the recruitment of different subtypes of DC, such as Langerhans cells, stromal DC-SIGN+ DCs and PDCs, associated with a significant proportion of mature DCs, acquiring a CCR7+ 'migratory' phenotype, indicate that they may play a pivotal role in the development of the inflammatory infiltrate that occurs typically in OLP.

## ROLE OF T CELLS IN LP & LM

**Takeuchi Y, Tohani I, Kaneda T *et al* (1988)**[61] analyzed immunohistochemically lymphocytes infiltrating OLP mucosal lesions, particularly in satellite cell necrosis (SCN) regions, and cytotoxic/suppressor T lymphocytes were predominant in seven of 10 OLP cases. Some carried IL-2 receptors, particularly in SCN. Keratinocytes of the mucosal lesions expressed both HLA-ABC and HLA-DR antigens. This suggests that activated cytotoxic/suppressor T lymphocytes may play a major role in cytotoxicity to keratinocytes as effector cells in OLP. HLA-DR+ and IL-1+ cells, vascular endothelial cells and clusters of spindle-shaped or oval cells, which had the same surface characteristics as a monocyte macrophage subset, were abundant, and together with migrating lymphocytes, might play a role in the immune response in mucosal lesions of OLP.

**Walsh LJ, Tseng PW, Savage NW *et al* (1989)**[65] studied that CD45R and CDw29 antigens are expressed on naive and primed helper T cell populations which serve in suppressor-inducer or helper-inducer functions, respectively. These antigens may also be expressed on epithelial cell subpopulations. Monoclonal antibodies reacting with T lymphocytes and LC were used to characterize the expression of CD45R and CDw29 antigens in OLP. Expression of CD45R was confined to intra-epithelial cells with either lymphocytic or dendritic morphology. They demonstrated the existence of intra-epithelial cells expressing antigens, which are functionally important in T cell responses, which may provide local immunoregulatory influences.

**Porter K, Klouda P, Scully C *et al* (1993)**[48] studied the frequencies of HLA in a group of 40 patients with OLP and compared it with those of healthy controls. Alterations in the frequencies of several HLA antigens were noted. An increase in HLA– Bw57 and decrease in frequency of HLA–DQ 1 were seen in LP. This suggest that LP may represent a heterogeneity of disease and that HLA – Bw57 may predispose a person to LP whereas HLA – DQ may be associated with resistance to it.

**Walton LJ**, **Macey MG**, **Thornhill MH *et al* (1998)**[66] studied that the circulating 'memory' subset (CD45RO+) of T-helper cells (CD4+) was increased from 49.1% in controls to 65.7% in patients (P=0.005), while the naive subset (CD45RA+), which was absent from control epithelium, comprised 24% of helper cells in OLP (P=0.016). Fewer LC expressed CD45RO in OLP than in controls (P=0.037) and all T-cell and LC counts were significantly raised in ICAM-1-expressing areas of epithelium. These data demonstrate changes in intra-epithelial T-lymphocyte and LC populations compared with normal oral mucosa and suggest there is selective recruitment in OLP.

**Rodriguez-Nunez I**, **Blanco-Carrion A**, **Garcia AG *et al* (2001)**[52] investigated possible immunologic differences between 26 patients with reticular OLP and 26 patients with atrophic-erosive OLP. The mean proportions of CD4+CD45RO+ and DR+ lymphocytes were significantly higher in patients with atrophic-erosive OLP than in patients with reticular OLP. These findings suggest that the two clinical types of OLP might have different immunopathogenic mechanisms.

**Zhou XJ, Sugerman PB, Savage NW *et al* (2002)**[73] investigated basement membrane (BM) disruption & distribution of mast cells and T cells in OLP using immunohistochemistry. The number of intra-epithelial CD8 T cells in region of BM

disruption was significantly greater than in regions of BM continuity $p<0.05$. The number of CD4 T cells in the epithelium and lamina propria of LP did not vary between regions of BM disruption and BM continuity. These data suggest a role for mast cells in epithelial BM disruption in LP. CD8 T cells may migrate through BM breaks to enter the OLP epithelium.

**Kawamura E**, **Nakamura S**, **Sasaki M *et al* (2003)**[30] proposed that T-cell receptor (TCR) in OLP is one of the most important steps to reveal the pathogenic antigen recognized by the T cells and thereby elucidate the pathogenesis and etiology of OLP. 7 patients with OLP, the TCR V beta gene usage was examined by polymerase chain reaction and single-strand conformation polymorphism analyses. The V beta families predominantly expressed in the biopsy specimens, the accumulation of T-cell clonotypes was observed in the majority of the V beta families including V beta 6, V beta 19and V beta 2. These results suggest that unique T-cell populations bearing V beta 2, V beta 6, or V beta 19 gene products tend to expand in OLP lesions as a consequence of *insitu* stimulation with a restricted epitope of either a nominal antigen on the MHC molecule for the majority of the V beta families, even if only in minor populations, or of a common superantigen for the minority of the V beta families.

**Khan A, Farah CS, Savage NW *et al* (2003)**[31] described the cell-mediated immune responses in OLP, which may be regulated by cytokines and their receptors. Immunohistochemistry and ELISA determined cytokine secretion in OLP. Majority of subepithelial and intra-epithelial mononuclear cells in OLP was CD8+ and some were found adjacent to degenerating keratinocytes. CD4+ cells were observed mainly in the deep lamina propria with occasional CD4+ cells close to basal keratinocytes.

Mononuclear cells expressed IFN-gamma in the superficial lamina propria and TNF-alpha adjacent to basal keratinocytes. TNF R1 was expressed by mononuclear cells and basal and suprabasal keratinocytes. These data suggest the development of a T helper$_1$ immune response that may promote CD8+ cytotoxic T cell activity in OLP.

**Sato M, Tokuda N, Fukumoto T *et al* (2006)**[55] studied that cGVHD is a common and serious complication after bone marrow transplantation (BMT). The immunohistopathological features of cGVHD compared with oral lichen planus (OLP) and healthy controls. Results showed that the infiltrations of CD4-positive T cells of cGVHD and OLP were significantly larger than those of the normal oral mucosa. A larger number of CD8-positive T cells were infiltrated in cGVHD and OLP compared with the normal oral mucosa. The difference in the number of CD4- and CD8-positive T cells between cGVHD and OLP was not significant. The infiltrations of LC (CD1a) in cGVHD and OLP were significantly larger than in the normal oral mucosa. The difference in the number of LC between cGVHD and OLP was not significant. It is suggested that LC and CD8-positive T cell may play a major role in the pathogenesis of the oral lichenoid lesions of cGVHD, and the immune response was inducted in OLP as well as the oral lichenoid lesion of cGVHD in this study.

# *Materials & Methods*

## Study design:

The study was designed to record the clinical details of patients with OLP & LM, and to study the LC & T cell population using immunohistochemical procedures.

## Study setting:

The study was conducted in the department of Oral and Maxillofacial Pathology, Ragas dental college and hospital, Chennai.

## Study subject:

15 cases of OLP & 15 cases of LM were selected. A predetermined clinical case sheet was used to record all the cases. Detailed case history including age, sex, occupation, past medical history and dental history with history of habits, drugs & traumas were recorded. This is followed by general examination & intraoral examination.

Clinically appearing bilateral white lesion having the characteristic Wickham's striae with or without dermatological manifestation was considered as LP. As the clinical features of LP & LM are similar, white lesions with bilateral distribution were categorized as LP and those with unilateral distribution as LM clinically. Informed consent was taken from all cases of OLP & LM prior to the biopsy procedure.

However, the final diagnosis was based on histopathological features in which dense band of inflammatory cells predominantly consists of lymphocytic infiltrate, confined to subepithelial region, hyperkeratosis and basal cell liquefaction[28].

The histopathological findings of LM differ from LP. In LM areas show mixed inflammatory cell response with lymphocytes, eosinophils and plasma cells, which is more diffuse & extends to deeper region[42].

**Controls:**

Biopsies from the 10 patients were taken from normal buccal mucosa adjacent to the site of surgery during the surgical removal of third molar or from patients who underwent orthodontic extractions after the informed consent *(Fig 5).* All the patients were in good general health and none of them had taken anti-microbial or anti-inflammatory drugs within the previous 3 months.

**Tissue specimens:**

Incisional biopsy of sufficient width and depth to ensure inclusion of connective tissue was taken from buccal mucosa. The tissue taken was immediately transferred to 10% buffered formalin for further processing. After adequate fixation paraffin blocks of tissue were made. From the blocks, 5-micron thick sections were cut and used for routine haematoxylin and eosin (H & E) staining and immunohistochemical staining using biotin streptavidin methods. (CD45RO and CD1a monoclonal antibody)

## IMMUNOHISTOCHEMISTRY (IHC):

### Armamentarium for IHC

1. Aluminum foil
2. APES coated slides
3. Autoclave
4. Beakers
5. Coplin jars
6. Cover slips
7. Cyclomixer
8. Electronic timer
9. Hot air oven
10. Light microscope
11. Measuring jar
12. Micropipettes
13. Pasteur pipettes
14. Rectangular steel trays with glass rods
15. Refrigerator
16. Microtome
17. Slide carrier
18. Slide warmer
19. Sterile gauze
20. Tooth forceps
21. Weighing machine (DHONA 200D)

## Reagents:

1. Distilled water
2. Laxbro solution
3. 1 N Hydrochloric acid
4. APES (3-amino propyl tri ethoxy silane)
5. Acetone
6. Xylene
7. Absolute alcohol
8. Alcohol 70%
9. Hydrogen peroxide 3%
10. Citrate buffer (pH 6)
11. Phosphate buffer saline (pH 7)
12. Hematoxylin
13. Ammonia
14. DPX

## Antibodies:

- DAKO ™ Monoclonal mouse anti human CD1a , Clone (01∅)
- DAKO ™ Monoclonal mouse anti human CD45RO, Clone (UCHL1)
- DAKO ™ LSAB 2 KIT/ HRP
    - Biotinylated Link
    - Streptavidin Peroxidase
    - Chromogen DAB (3 Diaminobenzidine Tetrahydrochloride)

Before taking the sections on to the slides, all the slides were APES coated. Precoating procedure of the slides was as follows:

**Pretreatment of the slides**

- The slides were first washed in tap water for few minutes
- They were then soaked in detergent solution for 1 hour
- After 1 hour, each slide was brushed individually using the detergent solution and then transferred to distilled water.
- Slides were washed in two changes of distilled water.
- Later slides were again washed in autoclaved distilled water.
- The slides were then immersed in 1 N HCL (100 ml HCL in 900 ml distilled water) overnight.
- The following day, slides were taken out of acid and washed in two changes of autoclaved distilled water.
- All the slides were then transferred to slide trays, wrapped in aluminium foil and baked in hot air oven for 4 hours at 180 degrees centigrade.

**APES (3 Amino propyl tri ethoxy silane) coating:**

Slides were first dipped in a coupling jar-containing acetone for 2 minutes

⬇

Dipped in APES for 5 minutes

⬇

Dipped in two changes of distilled water for 2 minutes

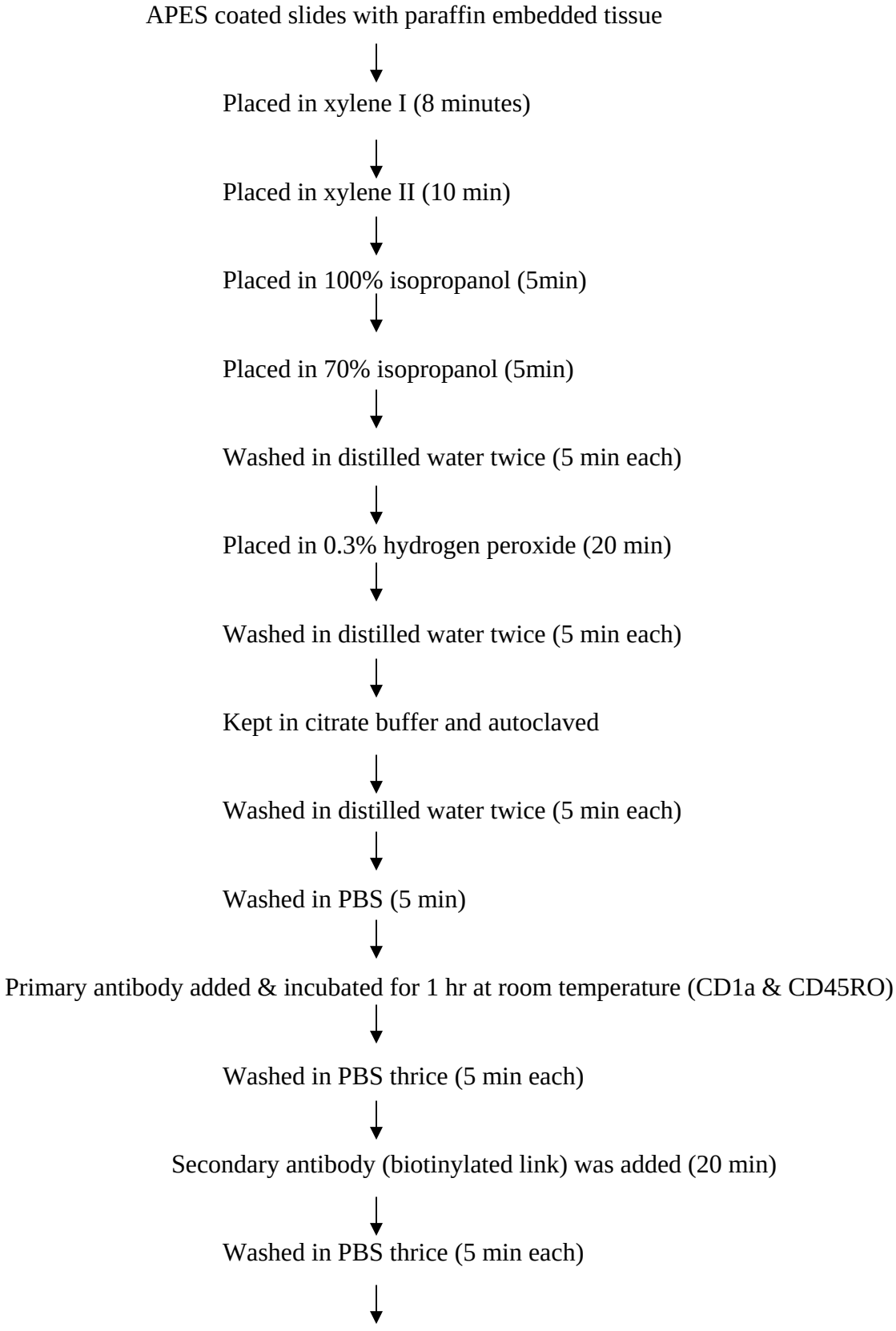
⬇

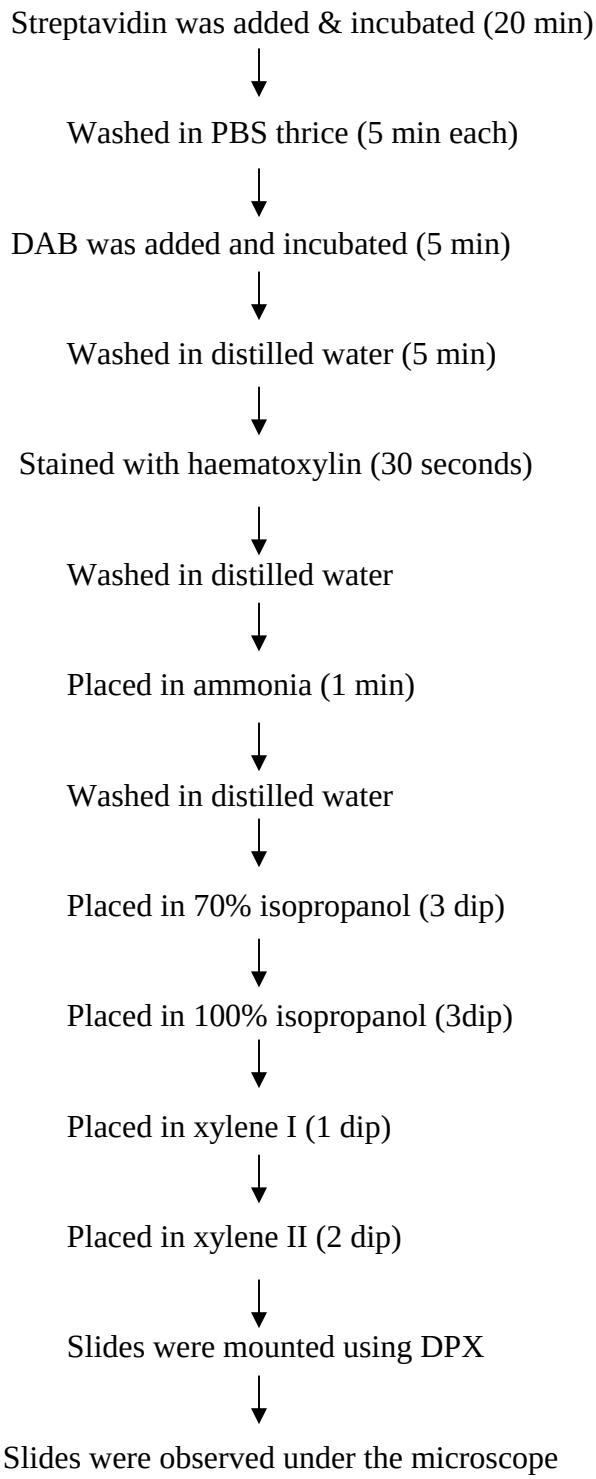
Slides were left to dry

**Preparation of paraffin sections**

After the slides were dry, tissue section of 0.5-micron thickness were made in a rotary manual microtome. The ribbons of tissue section were transferred onto the APES coated slide from the tissue float both such that two tissue bits come on to the slide with a gap in between. One of the tissue sections was labeled positive (P) and the other negative (N). Circles were drawn with a glass-marking instrument around the tissue, so that the antibodies were localized in the area of interest.

**IHC procedure flow chart: CD1a & CD45RO Antibody**




Streptavidin was added & incubated (20 min)
Washed in PBS thrice (5 min each)
DAB was added and incubated (5 min)
Washed in distilled water (5 min)
Stained with haematoxylin (30 seconds)
Washed in distilled water
Placed in ammonia (1 min)
Washed in distilled water
Placed in 70% isopropanol (3 dip)
Placed in 100% isopropanol (3dip)
Placed in xylene I (1 dip)
Placed in xylene II (2 dip)
Slides were mounted using DPX
Slides were observed under the microscope

**Immunohistochemistry procedure**

The slides with tissue sections were treated with two changes of xylene to remove paraffin wax. They were put in descending grades of alcohol and then rehydrated with water. Slides were then treated with 3% hydrogen peroxide for 20 minutes to quench endogenous peroxidase activity of cells that would otherwise result in nonspecific staining. The slides were then put in two changes of distilled water.

The slides were then transferred to citrate buffer and autoclaved for antigen retrieval at 15 lbs pressure for 15 minutes. The slides were then washed in two changes of distilled water. Then the slides were dipped in PBS for 5 minutes, and then wiped carefully with gauze to remove excess PBS. Circles were drawn around the tissues, so that the antibodies added later on do not spread and are restricted to the circle. The primary antibody CD45RO & CD1a (DAKO) was added separately to P tissue on the slide and PBS was added to the N tissue. The petridish containing the slides was kept at room temperature for 1 hour.

The sections taken out were washed in three changes of cold PBS for 5 minutes each to remove the excess antibody. Then the slides were wiped carefully without touching the tissue section to remove excess PBS. Then a drop of Biotinylated link from the secondary antibody kit (DAKO LSAB 2KIT) was added on both the sections and the slides incubated for 20 minutes. Later the slides were washed in three changes of cold PBS for 5 minutes each. The slides were again wiped carefully without touching the tissue section to remove excess PBS. Then a drop of Streptavidin from the secondary antibody kit (DAKO LSAB 2KIT) was added on both the sections and the slides

incubated for 20 minutes. The sections were then washed in 3 changes of cold PBS for 5 minutes each. Then the slides were again wiped carefully to remove excess PBS.

Then a drop of freshly prepared DAB (3'– Diamino benzidine tetra hydrochloride – a substrate chromogen) was added on to both sections. Slides were then washed in running distilled water to remove excess DAB and counter stained with haematoxylin. The slides were dipped in ammonia for one minute for blueing. Then the slides were transferred to 70% alcohol, 100% alcohol and two changes of xylene. The tissue sections were mounted with DPX. Slides were then observed under the microscope. Throughout the procedure care was taken not to dry the tissues.

Analysis of CD1a & CD45RO expression was done by evaluating the positive cells. CD1a positive cell showed staining pattern as membranous and weakly cytoplasmic. CD45RO positive cell showed membranous staining (as recommended by product specification).

Analysis of CD1a expression by evaluating the labeling index (LI) for each slide was calculated by dividing the number of positive cells by the total number of cells counted in the slide and expressed as percentage. A total of thousand cells were counted in each slide.

$$LI = \frac{\text{Number of positive cells}}{1000} \times 100$$

Analysis of CD1a & CD45RO expression was done by evaluating the staining intensity. Slides were assessed for mild (+), moderate (++), intense (+++), or no expression (-). 2 investigators using Kappa statistics compared CD1a and CD45RO antibody staining intensity between the study groups. Kappa statistics was done for interpretation of the inter-observer variation.

# *Statistical Analysis*

## STATISTICAL ANALYSIS

Data entry and analysis was performed using SPSS version 10.0.5®. Mean LI and standard deviation was calculated to assess CD1a expression and percentage of intensity was calculated to assess CD1a & CD45RO expression.

- Student t-test was done to compare the descriptive variables between the study groups.
- Analysis of variance (ANOVA) was done to compare the mean LI of CD1a between LP, LM and NM.
- Bonferroni test of multiple comparison test was done to compare the LI of CD1a between LP, LM and NM.
- Kappa statistics for the inter-observer variation of CD1a and CD45RO antibody staining intensity between the study groups.
- Chi-square test was done to compare the percentage expression of CD1a & CD45RO between LP, LM and NM.

# *Results*

This study was to compare the clinical and histopathological features of LP and LM and evaluate LC and T cells number and activity using monoclonal antibody CD1a and CD45RO.

Of the 15 cases of LP 10 were females and 5 were males. The mean age was 36 years ± 17 for males and 44 years ± 12 for females. All 15 cases involved the buccal mucosa. In addition to buccal mucosa, the alveolar mucosa was affected in 2 cases and gingiva in 1 case. 12 cases showed a bilateral distribution and 3 cases had a unilateral distribution. (***Table 1***).

Of the 15 cases of LM 10 were females and 5 were males. The mean age was 38 years ± 17 for males and 40 years ± 11 for females. 9 out of 15 cases occurred in buccal mucosa, 5 cases had lesions in tongue, 3 cases in alveolar mucosa and 4 cases in gingiva. 1 case showed a bilateral distribution and all other 14 cases had a unilateral distribution (***Table 1***).

Of the 10 cases of NM 3 were females and 7 were males. The mean age was 40 years ± 15 for males and 24 years ± 5 for females. In 7 out of 10 cases, biopsy was taken from buccal mucosa and 3 cases from gingiva as control. (***Table 1***)

Among the 15 cases of LM, 8 (53.33%) cases had drug history of NSAID drug intake and 3 (20%) cases had antihypertensive drugs, 2 (13.33%) cases had hypoglycemic drug and 1 (6.67%) case had tetracycline. 1 case (6.67%) had multiple amalgam fillings (***Table 2***).

Age and gender analysis among the study group (LP, LM & NM) were not statistically significant (LP $p = 0.27$, LM $p = 0.15$, NM $p = 0.43$) (***Table 3***).

**CD1a antibody Mean Labeling Index between the study groups:**

The Mean Labeling Index in basal layer of LP was 5.31 with a standard deviation (SD) of 2.26 *(Fig 6 & 7)*; in LM it was 2.36 with a SD of 2.17 *(Fig 8 & 9)* and in NM it was 0.87 with a SD of 0.65 *(Fig 10 & 11)*. The difference in mean LI between the study groups for basal layer was statistically significant (p = 0.00) (***Table4, graph 1***).

The Mean Labeling Index in supra basal layer of LP was 6.36 with a standard deviation (SD) of 3.24; in LM it was 3.54 with a SD of 2.70 and in NM it was 0.80 with a SD of 0.44. The difference in mean LI between the study groups for supra basal layer was statistically significant (p = 0.00) (***Table4, graph 1***).

The Mean Labeling Index in the connective tissue of LP was 62.67 with a standard deviation (SD) of 43.92; in LM it was 18.0 with a SD of 10.99and in NM it was 5.30 with a SD of 2.67.The difference in mean positive cells between the study groups for connective tissue layer was statistically significant (p = 0.00) (***Table4, graph 2***).

**Mean difference in CD1a antibody Mean Labeling Index between the study groups (Bonferroni Test):**

The Mean difference in Mean Labeling Index in basal layer of LP and LM was 2.96 and it was statistically significant (p = 0.001); in LP and NM was 5.54 and it was statistically significant (p = 0.00); in LM and NM was 1.49 and it was statistically not significant (p = 0.21).

The Mean difference in Mean Labeling Index in supra basal layer of LP and LM was 2.82 and it was statistically significant (p = 0.016); in LP and NM was 5.55 and it was statistically significant (p = 0.000); LM and NM was 2.73 and it was statistically significant (p = 0.043).

The Mean difference in Mean Labeling Index in connective tissue layer of LP and LM was 44.67 and it was statistically significant (p = 0.000); in LP and NM was 57.37 and it was statistically significant (p = 0.00); in LM and NM was 12.70 and it was statistically not significant (p = 0.815) (***Table 5***).

2 investigators using Kappa statistics compared CD1a and CD45RO antibody staining intensity between the study groups. Kappa statistics for the inter observer variation was between 0.6-0.8, and the agreement between the 2 evaluators was statistically significant in all the layers (p < 0.05) (***Table 6***).

**CD1a antibody staining intensity between the study groups:**

The staining intensity in the basal layer of LP, 2(13.3 %)cases showed no staining, 8(53.3%) cases mild staining, 1(6.7%) case moderate and 4(4.0%) cases were intensely stained. In LM, 3(20%)cases showed no staining, 9(60 %) cases mild staining, 3(20%) cases were moderately stained. In NM, 1(10 %) case showed no staining, 8(80%) cases mild staining and 1(10%) case was moderately stained. The difference in staining intensity pattern between the study groups was not statistically significant (p = 0.176) (***Graph3***)

In the supra basal layer of LP, 3(20%) cases showed no staining, 4(26.7 %) cases mild staining, 7(46.7%) cases moderate staining and 1(6.7%) case was intensely stained. In LM, 2(13.3%) cases showed no staining, 10(66.7%) cases mild staining and 3(20%) cases were moderately stained. In NM, 2(20%) cases showed no staining, 4(40%) cases each showed mild and moderate staining. The difference in staining intensity pattern between the study groups was not statistically significant (p = 0.39) (***Graph 4***).

In the connective tissue of LP, 5(33.3%) cases showed mild staining, 7(46.7%) cases moderate and 3(20%)cases were intensely stained. In LM, 3(20%) cases showed no staining, 5(33.3%) cases mild staining and 7(46.7%) cases were moderately stained. In NM, 4(40%) cases showed no staining and 6(60%) cases were showed mild staining. The difference in staining intensity pattern between the study groups was statistically significant (p = 0.011) (***Graph 5***).

The staining intensity pattern of CD1a antibody of basal and suprabasal layers of epithelium and connective tissue is given in (***Table 7, Graph 6***).

**CD 45 RO antibody mean percentage staining intensity between the study groups:**

In the connective tissue of LP, 1(6.7%) case showed mild staining, 6(40%) cases moderate and 8(53.3%) cases showed intense staining *(Fig 12 & 13).* In LM, 5(33.3%) cases showed mild staining, 7(46.7%) cases moderate staining and 3(20%) cases were intensely stained *(Fig 14 & 15).* In NM, 1(10%) case showed no staining, 6(60%) cases mild staining and 3(30%) cases were moderately stained *(Fig 16 & 17).* The difference in staining intensity pattern between the study groups was statistically significant (p= 0.014) (***Table 8, Graph 7***).

**CD1a and CD45RO antibody staining intensity between the study groups:**

In the connective tissue, the difference in staining intensity pattern between the study groups was not statistically significant. ***(Table 9, Graph 8).***

# *Tables & Graphs*

## EVALUATION OF T CELLS & LANGERHANS CELL IN LICHEN PLANUS & LICHENOID MUCOSITIS

### Table 1: Age, gender and site, distribution among the study groups

| Lesion | Sex | | Age | | Site | | | | Distribution | |
|---|---|---|---|---|---|---|---|---|---|---|
| | M | F | M | F | Buccal mucosa | Tongue | Alveolar mucosa | Gingiva | Bilateral | Unilateral |
| **Lichen planus** | 5 | 10 | 36 ± 17 | 44 ±12 | 15 | - | 2 | 1 | 12 | 3 |
| **Lichenoid mucositis** | 5 | 10 | 38 ± 17 | 40 ±11 | 9 | 5 | 3 | 4 | 1 | 14 |
| **Normal mucosa** | 7 | 3 | 40 ± 15 | 24 ± 5 | 7 | - | - | 3 | - | - |

### Table 2: Systemic drug intake and contact allergens among the LM study groups

| Drug & contact allergens | n (15) | % |
|---|---|---|
| NSAIDS | 8 | 53.33 |
| Antihypertensive drug | 3 | 20.00 |
| Hypoglycemic drug | 2 | 13.33 |
| Antibiotic (Tetracycline) | 1 | 6.67 |
| Multiple Amalgam fillings | 1 | 6.67 |

**Table 3: Age & gender distribution between the study groups**

| Age groups | Male | | Female | | p value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| **Lichen Planus** | | | | | |
| <25 | 1 | 25.0 | 2 | 18.2 | |
| 25 - 34 | - | - | - | - | |
| 35 – 44 | - | - | 4 | 36.4 | .270 |
| 45 – 54 | 3 | 75.0 | 3 | 27.3 | |
| >55 | - | - | 2 | 18.2 | |
| **Lichenoid Mucositis** | | | | | |
| <25 | 1 | 20.0 | - | - | |
| 25 - 34 | 2 | 40.0 | 3 | 30.0 | |
| 35 – 44 | - | - | 5 | 50.0 | .159 |
| 45 – 54 | 1 | 20.0 | - | - | |
| >55 | 1 | 20.0 | 2 | 20.0 | |
| **Normal Mucosa** | | | | | |
| <25 | 1 | 12.5 | 1 | 50.0 | |
| 25 -34 | 2 | 25.0 | 1 | 50.0 | |
| 35 – 44 | 2 | 25.0 | - | - | .439 |
| 45 – 54 | 3 | 37.5 | - | - | |
| >55 | - | - | - | - | |

**Table 4: CD1a antibody mean labeling index between the study groups**

| | n | Mean | p value |
|---|---|---|---|
| **Basal cells** | | | |
| Lichen planus | 15 | 5.31 ± 2.26 | |
| Lichenoid mucositis | 15 | 2.36 ± 2.17 | .000** |
| Normal mucosa | 10 | 0.87 ± 0.65 | |
| **Supra basal cells** | | | |
| Lichen planus | 15 | 6.36 ± 3.24 | |
| Lichenoid mucositis | 15 | 3.54 ± 2.70 | .000** |
| Normal mucosa | 10 | 0.80 ± 0.44 | |
| **Connective tissue •** | | | |
| Lichen planus | 15 | 62.67 ± 43.92 | |
| Lichenoid mucositis | 15 | 18.00 ± 10.99 | .000** |
| Normal mucosa | 10 | 5.30 ± 2.67 | |

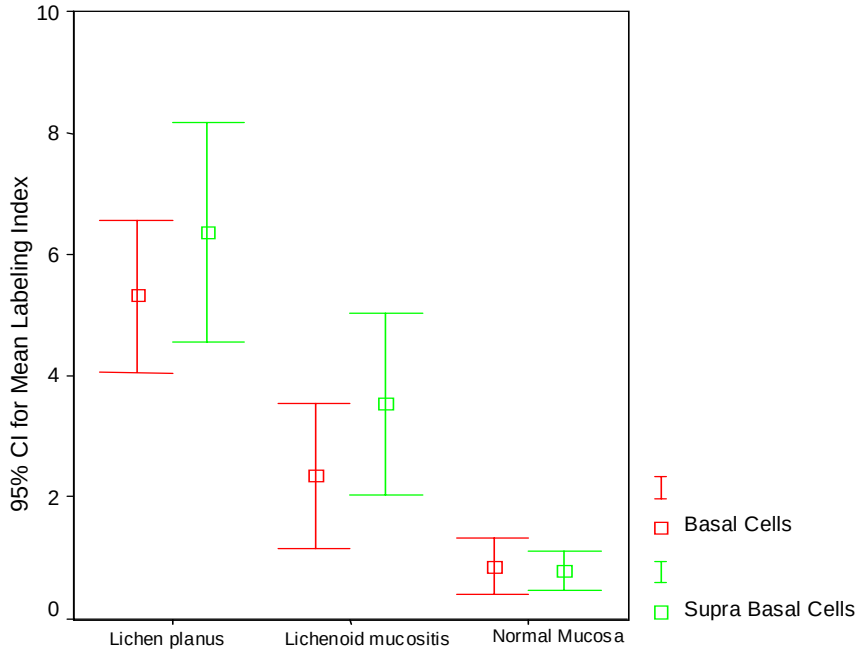
• Mean positive cells ** $p < 0.01$ Statistically significant

**Table 5: Mean difference in CD1a antibody mean labeling index between the study groups**

| Study groups | | Mean difference | p value |
|---|---|---|---|
| ***Basal layer*** | Lichen planus<br>Lichenoid mucositis | 2.96 | .001** |
| | Lichen planus<br>Normal mucosa | 4.45 | .000** |
| | Lichenoid mucositis<br>Normal mucosa | 1.49 | 210 |
| ***Supra Basal layer*** | Lichen planus<br>Lichenoid mucositis | 2.82 | .016* |
| | Lichen planus<br>Normal mucosa | 5.55 | .000** |
| | Lichenoid mucositis<br>Normal mucosa | 2.73 | .043* |
| ***Connective tissue*** | Lichen planus<br>Lichenoid mucositis | 44.67 | .000** |
| | Lichen planus<br>Normal mucosa | 57.37 | .000** |
| | Lichenoid mucositis<br>Normal mucosa | 12.70 | .815 |

** $p < 0.01$ Statistically significant
* $p < 0.05$ Statistically significant

**Graph 1: CD1a antibody mean labeling index of basal & suprabasal epithelial layer between the study groups**




**Graph 2: CD1a antibody mean positive cells of connective tissue between the study groups**

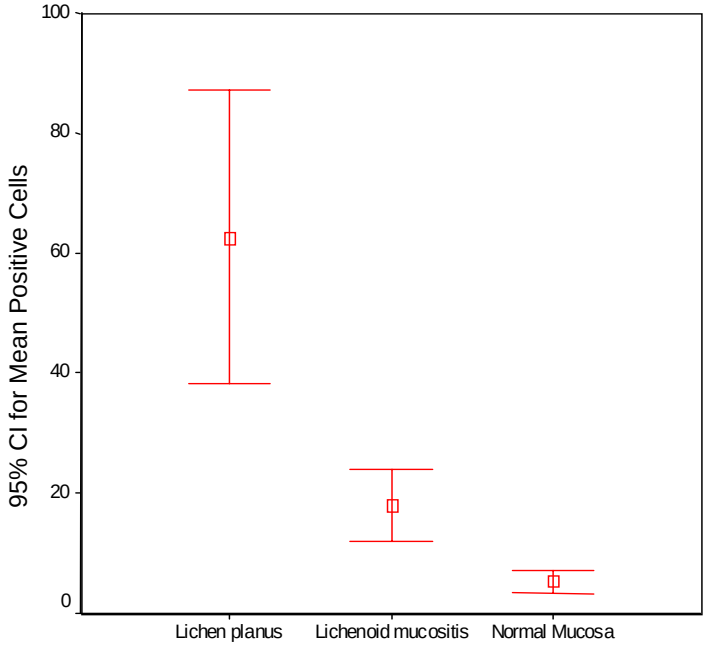
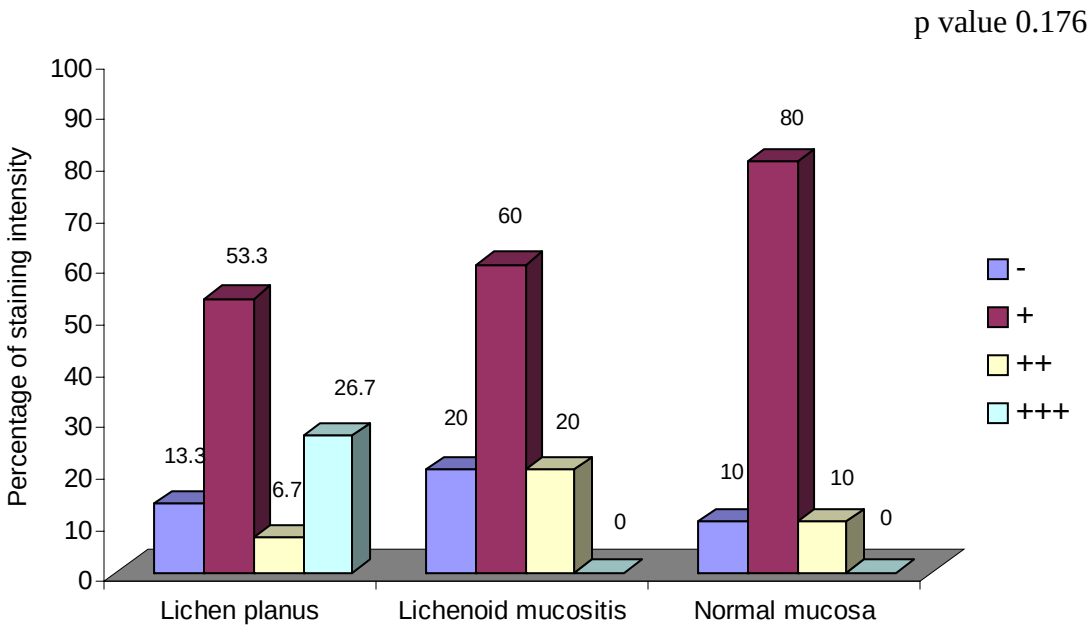


**Table 6: Kappa statistics - For CD1a & CD45 RO antibody staining intensity**

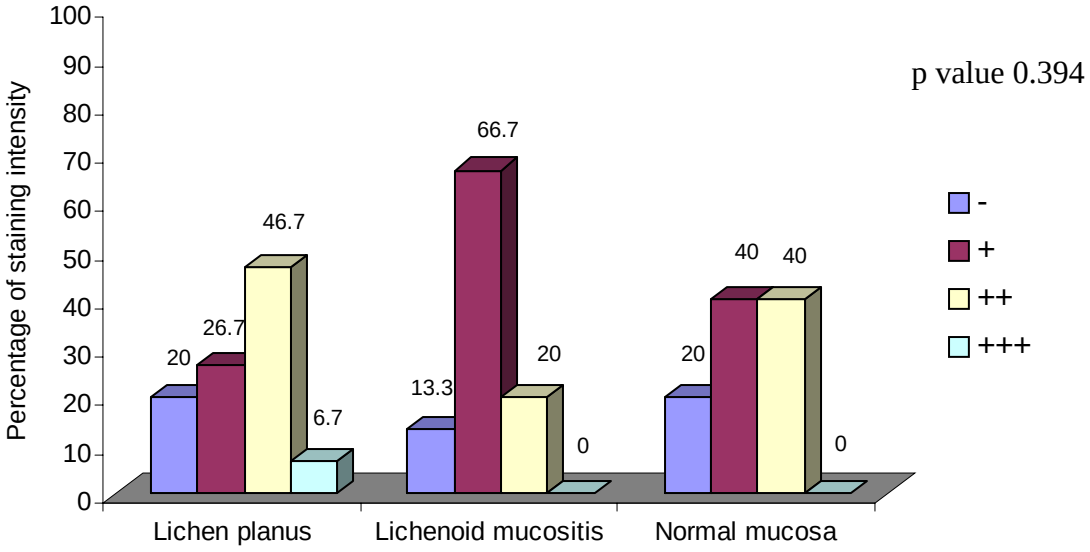
| | | Lichen planus | Lichenoid mucositis | Normal mucosa |
|---|---|---|---|---|
| **CD1a** | Basal | 0.71 | 0.73 | 0.58 |
| | Supra basal | 0.68 | 0.57 | 0.69 |
| | Connective tissue | 0.67 | 0.68 | 0.78 |
| **CD45RO** | Connective tissue | 0.75 | 0.78 | 0.64 |

Agreement between two evaluators is statistically significant in all the layers $p < 0.05$

**Graph 3: CD1a antibody staining intensity of the basal layer between the study groups**




**Graph 4: CD1a antibody staining intensity of the supra basal Layer between the study groups**



**Graph 5: CD1a antibody staining intensity of the connective tissue between the study groups**

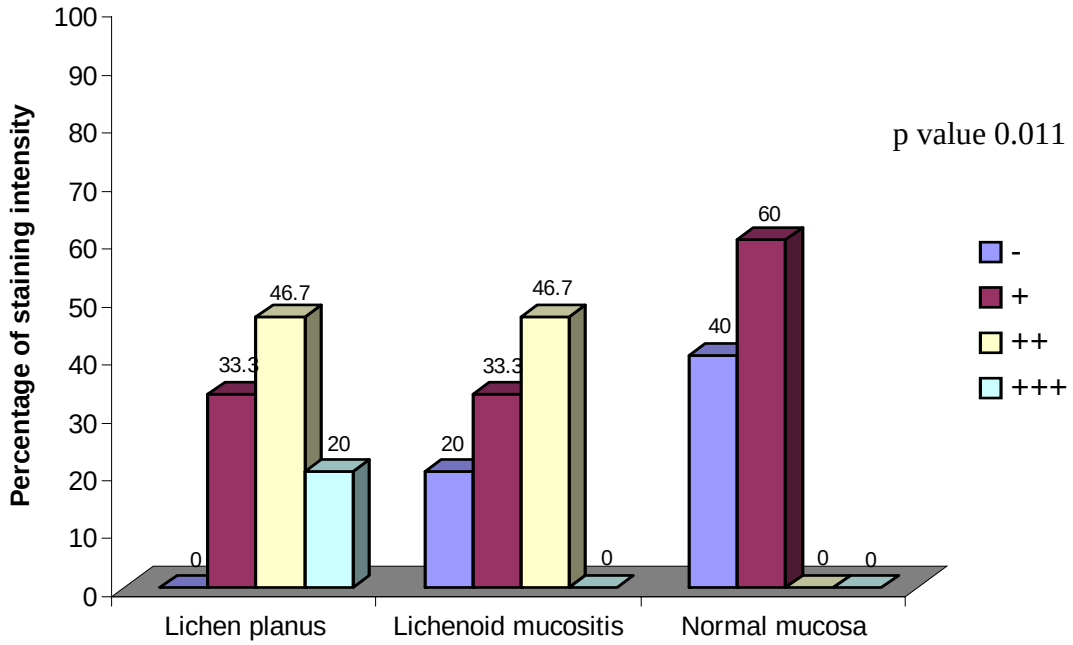


**Table 7: CD1a antibody staining intensity of the basal, supra basal layer & connective tissue between the study groups**

| Lesion | Basal n (%) | | | | Supra basal n (%) | | | | Connective tissue n (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | - | + | ++ | +++ | - | + | ++ | +++ | - | + | ++ | +++ |
| **Lichen planus (n 15)** | 2 (13.3%) | 8 (53.3%) | 1 (6.7%) | 4 (26.7%) | 3 (20%) | 4 (26.7%) | 7 (46.7%) | 1 (6.7%) | - | 5 (33.3%) | 7 (46.7%) | 3 (20%) |
| **Lichenoid mucositis (n 15)** | 3 (20%) | 9 (60%) | 3 (20%) | - | 2 (13.3%) | 10 (66.7%) | 3 (20%) | - | 3 (20%) | 5 (33.3%) | 7 (46.7%) | - |
| **Normal mucosa (n 10)** | 1 (10%) | 8 (80%) | 1 (10%) | - | 2 (20%) | 4 (40%) | 4 (40%) | - | 4 (40%) | 6 (60%) | - | - |
| **p value** | .176 | | | | .394 | | | | .011* | | | |

*** p < 0.05 Statistically significant**
**- Negative**
**+ Mild**
**++ Moderate**
**+++ Intense**

**Graph 6: CD1a antibody staining intensity of the basal, supra basal layer & connective tissue between the study groups**

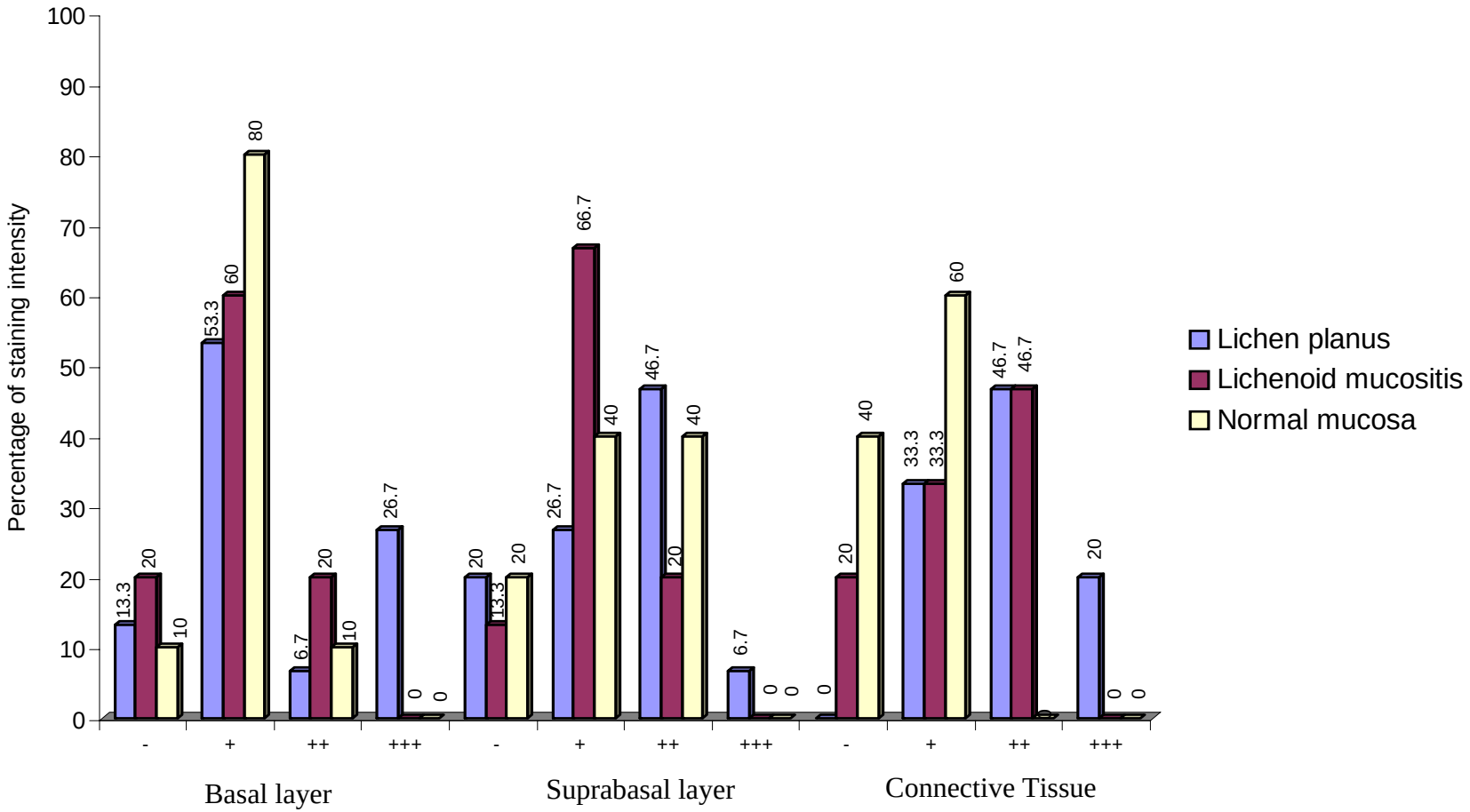


**Table 8: CD45RO antibody staining intensity of the connective tissue between the study groups.**

| Lesion | Connective tissue n (%) | | | | p value |
|---|---|---|---|---|---|
| | - | + | ++ | +++ | |
| **Lichen planus (n 15)** | - | 1 (6.7%) | 6 (40.0%) | 8 (53.3%) | .014* |
| **Lichenoid mucositis (n 15)** | - | 5 (33.3%) | 7 (46.7%) | 3 (20.0%) | |
| **Normal mucosa (n 10)** | 1 (10.0%) | 6 (60.0%) | 3 (30.0%) | - | |

* $p < 0.05$ Statistically significant

**Graph 7: CD45RO antibody staining intensity in connective tissue between the study groups**

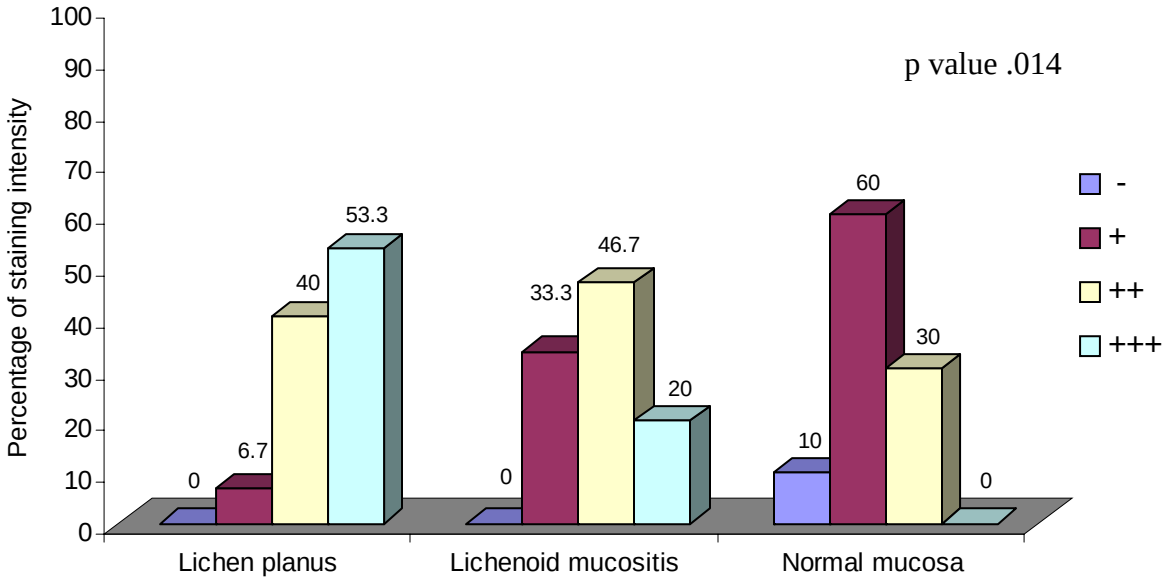
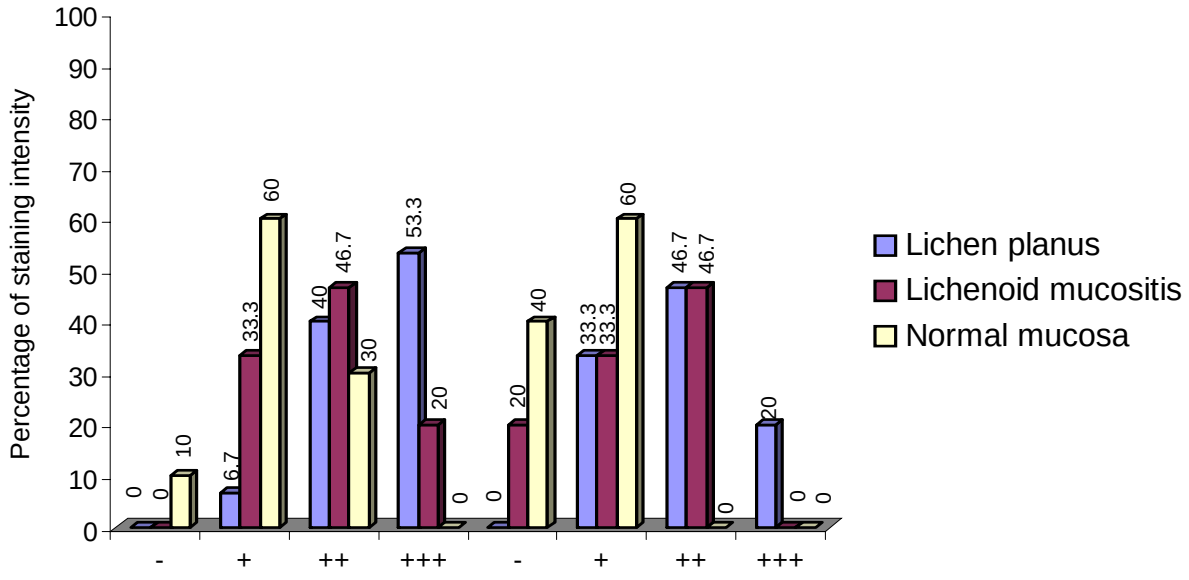


**Table 9: CD45RO & CD1a antibody staining intensity in connective tissue between the study groups**

| Lesion | CD45RO n (%) | | | | CD1a n (%) | | | | p value |
|---|---|---|---|---|---|---|---|---|---|
| | - | + | ++ | +++ | - | + | ++ | +++ | |
| **Lichen planus** (n 15) | 0 | 1 (6.7%) | 6 (40%) | 8 (53.3%) | 0 | 5 (33.7%) | 7 (46.7%) | 3 (20%) | .081 |
| **Lichenoid mucositis** (n 15) | 0 | 5 (33.3%) | 7 (46.7%) | 3 (20%) | 3 (20%) | 5 (33.3%) | 7 (46.7%) | 0 | .112 |
| **Normal mucosa** (n 10) | 1 (10%) | 6 (60%) | 3 (30%) | 0 | 4 (40%) | 6 (60%) | 0 | 0 | .091 |

**Graph 8: CD45RO & CD1a antibody staining intensity in connective tissue between the study groups**



# *Photomicrographs*

**Figure 1 Lichen Planus**

**Figure 2 Lichenoid Mucositis**

**Figure 3 Photomicrograph of Lichen Planus H&E (10x)**

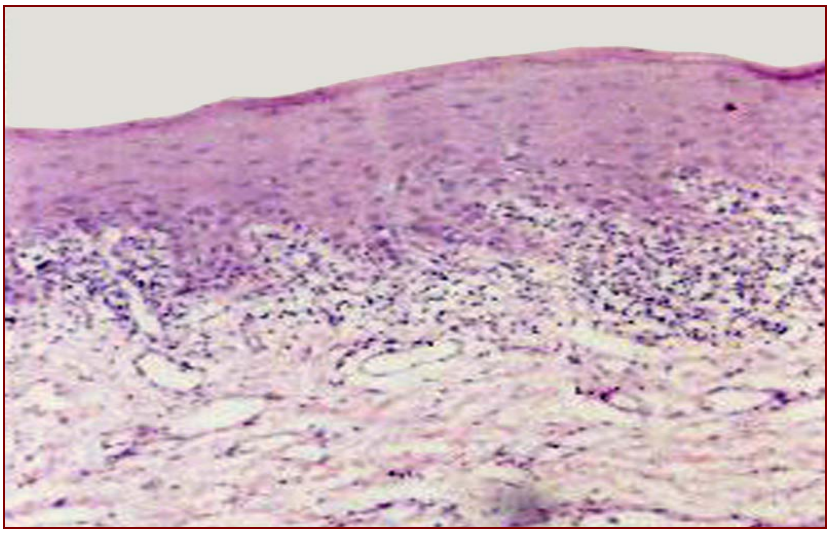


**Figure 4 Photomicrograph of Lichenoid Mucositis H&E (10x)**

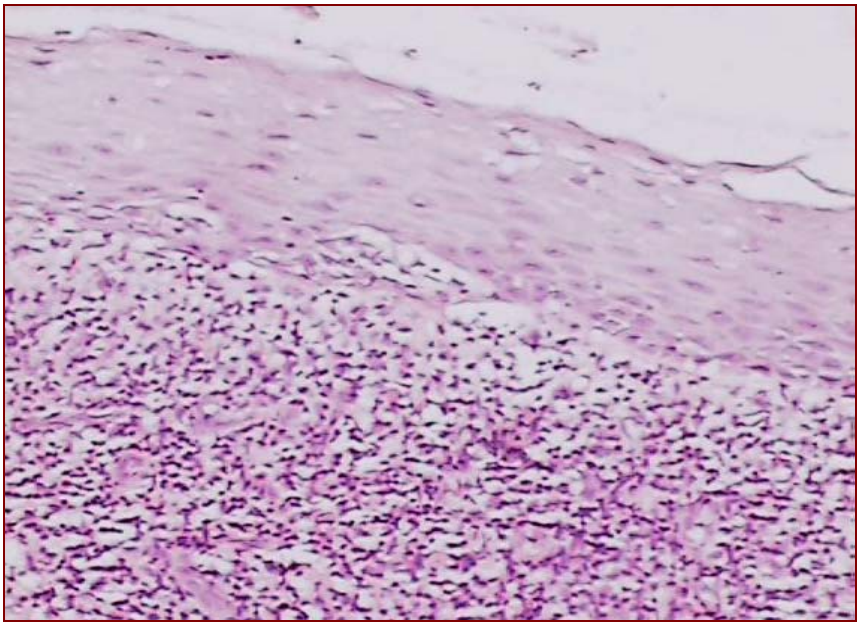


**Figure 5 Photomicrograph of Normal Mucosa H&E (10x)**

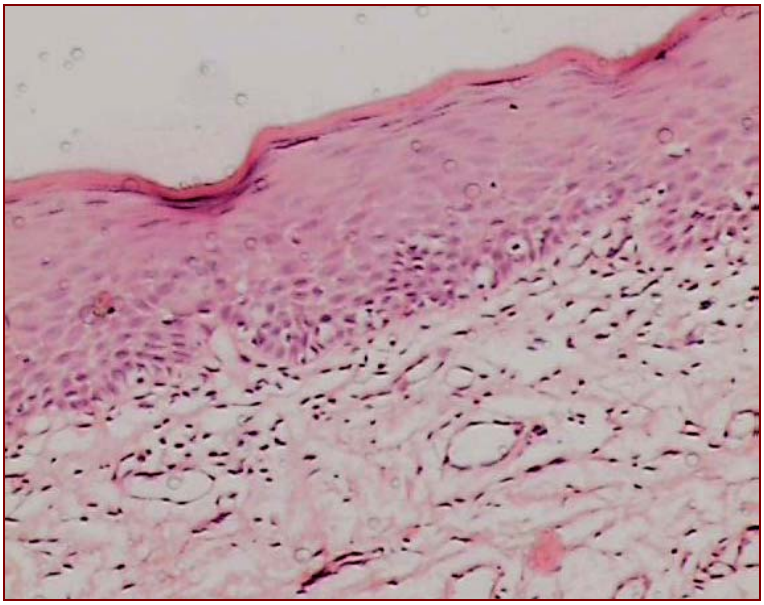


**Figure 6 Photomicrograph of CD1a expression in Lichen Planus (10x)**

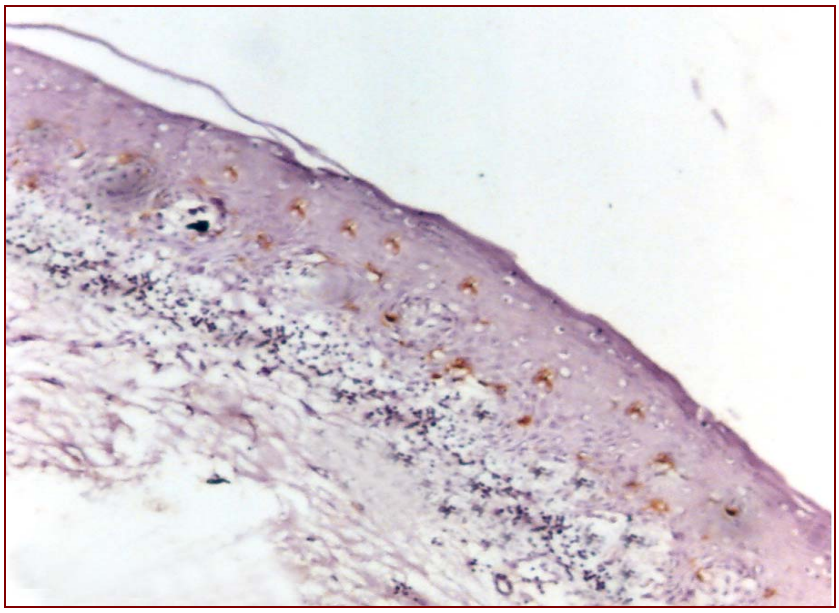


**Figure 7 Photomicrograph of CD1a expression in Lichen Planus (40x)**

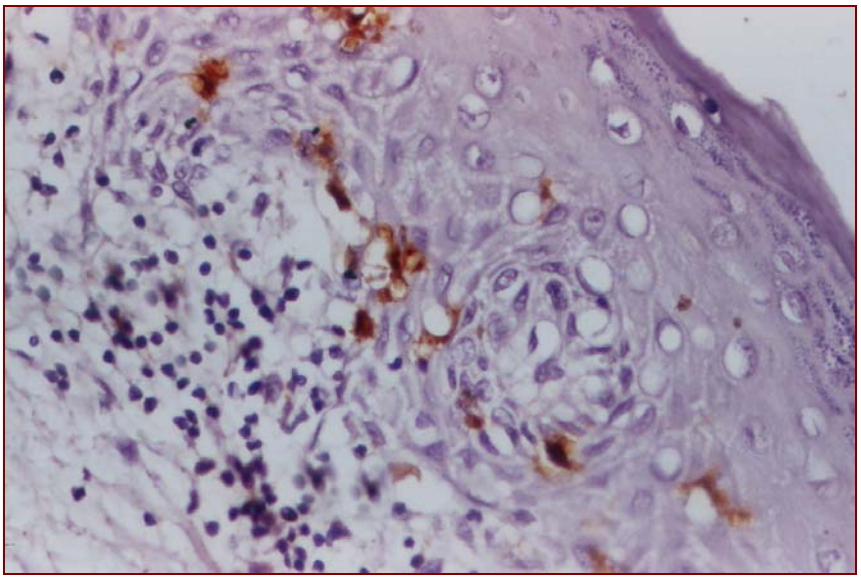


**Figure 8 Photomicrograph of CD1a expression in Lichenoid Mucositis (10x)**

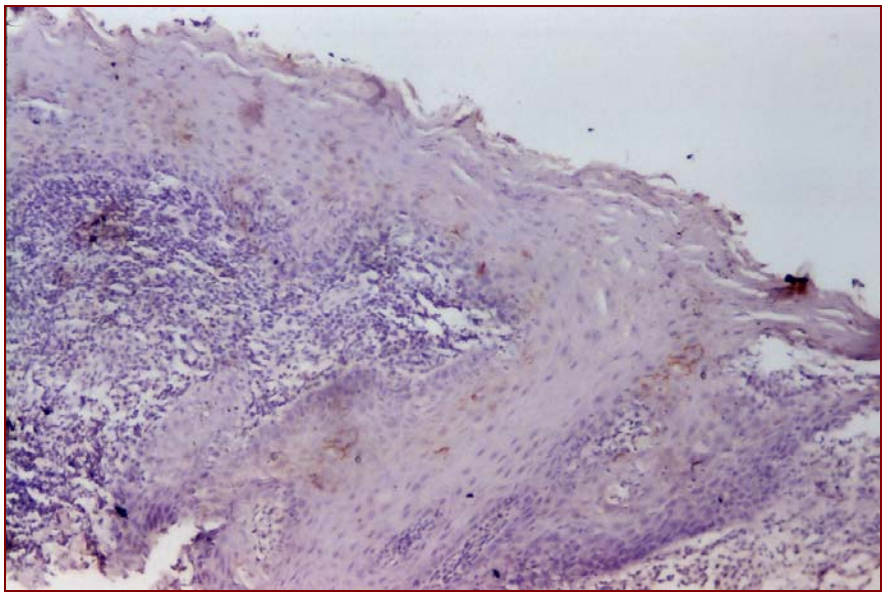


**Figure 9 Photomicrograph of CD1a expression in Lichenoid Mucositis (40x)**

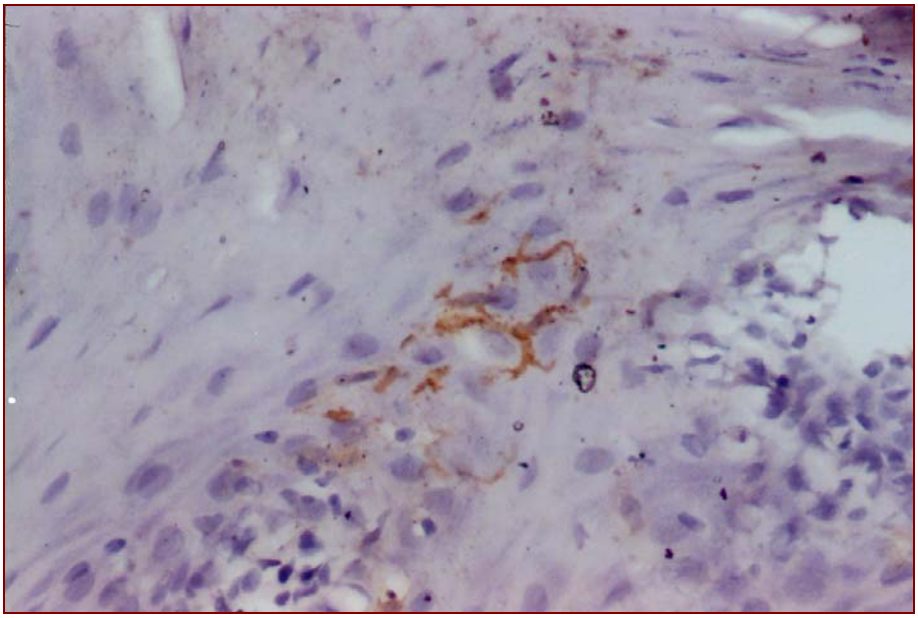


**Figure 10 Photomicrograph of CD1a expression in Normal Mucosa (10x)**

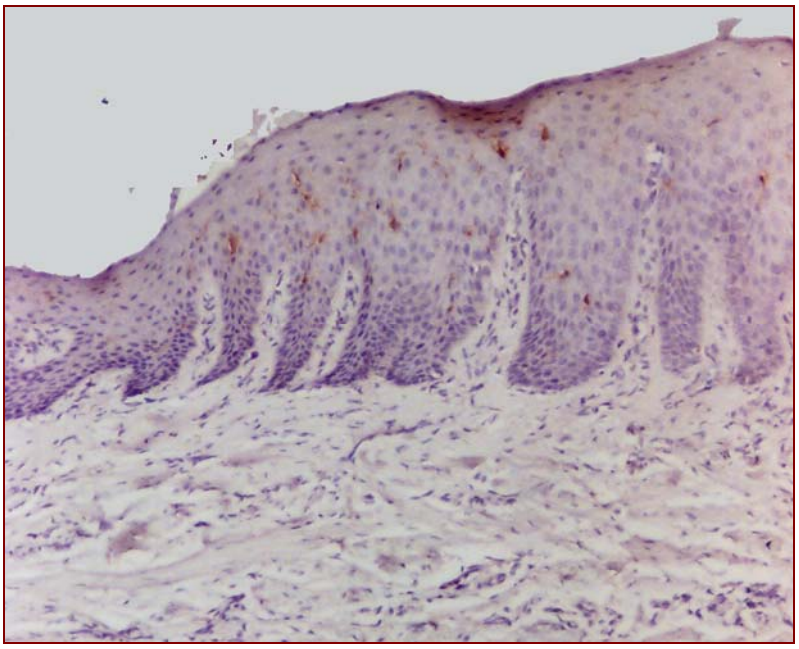


**Figure 11 Photomicrograph of CD1a expression in Normal Mucosa (40x)**

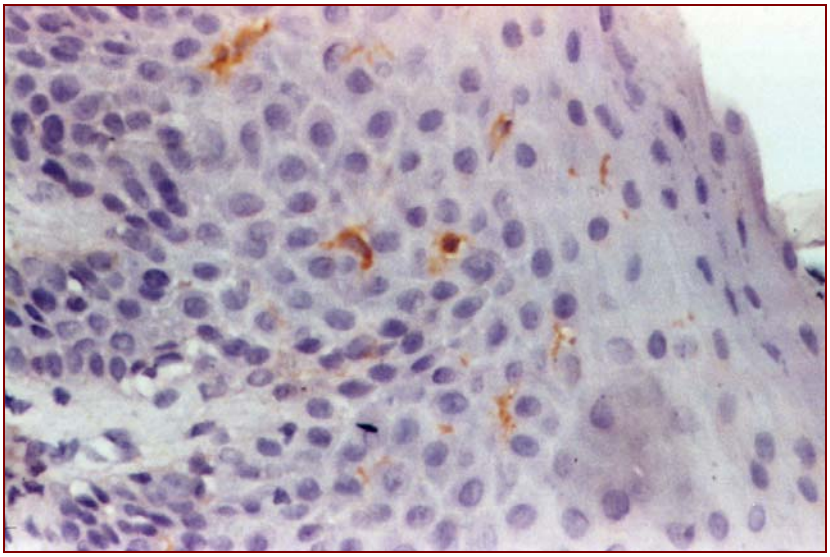


**Figure 12 Photomicrograph of CD45RO expression in Lichen Planus (10x)**

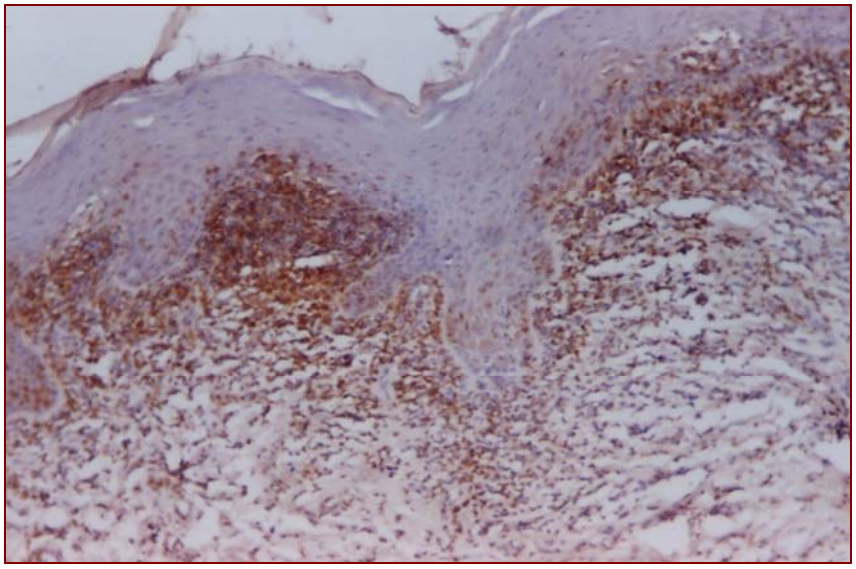


**Figure 13 Photomicrograph of CD45RO expression in Lichen Planus (40x)**

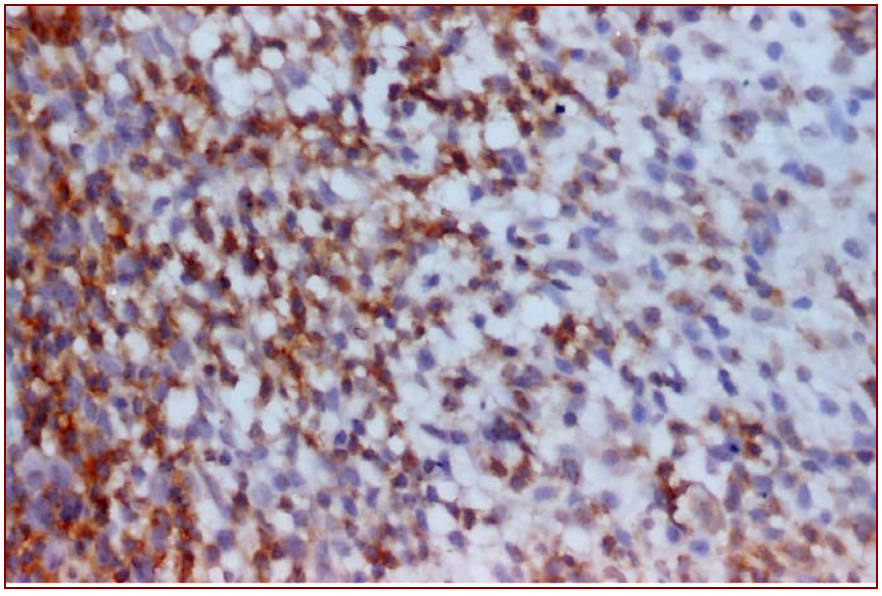


**Figure 14 Photomicrograph of CD45RO expression in Lichenoid Mucositis (10x)**

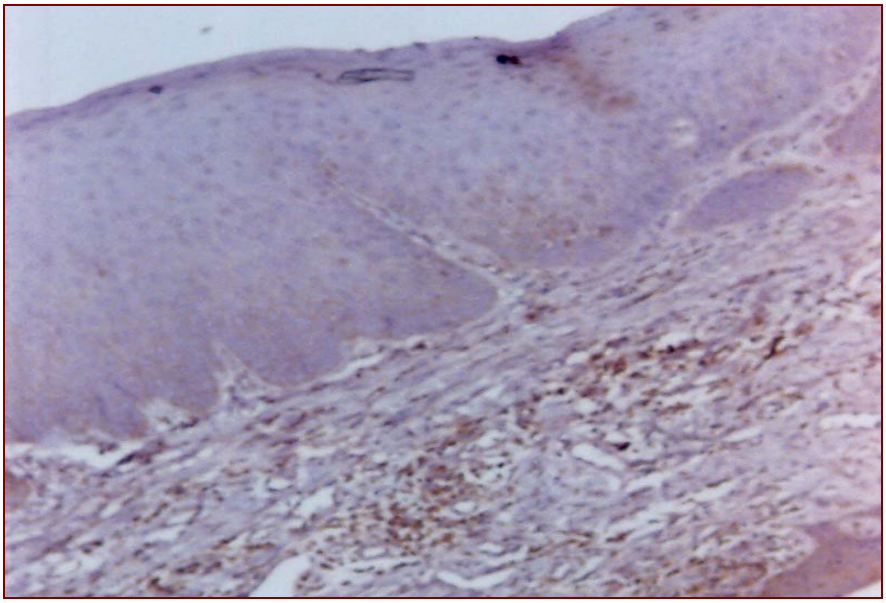


**Figure 15 Photomicrograph of CD45RO expression in Lichenoid Mucositis (40x)**

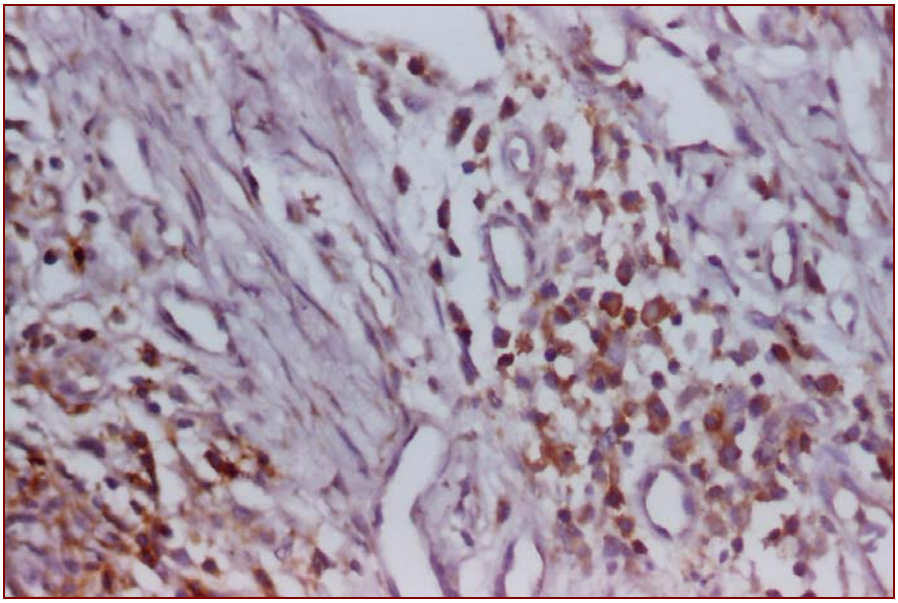


**Figure 16 Photomicrograph of CD45RO expression in Normal Mucosa (10x)**

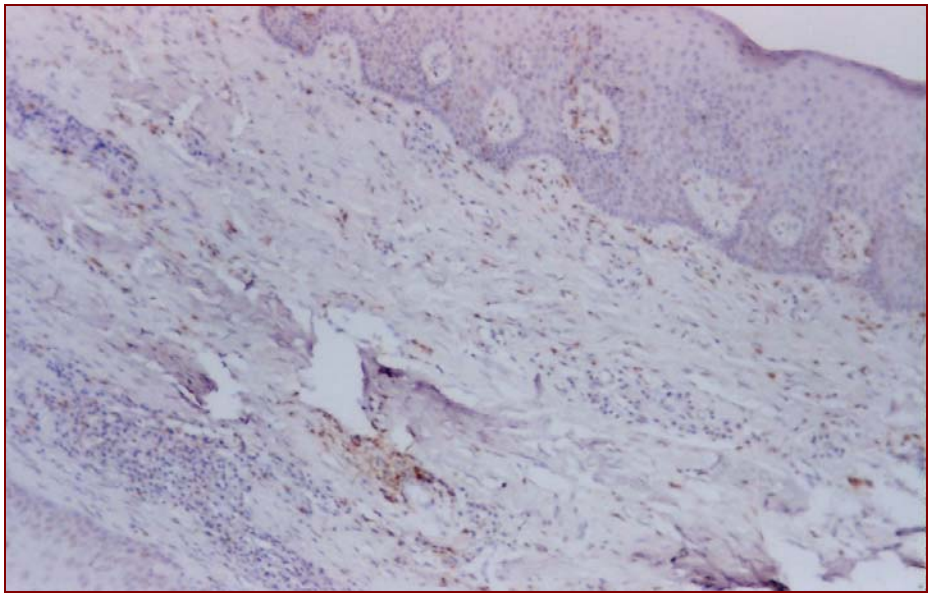
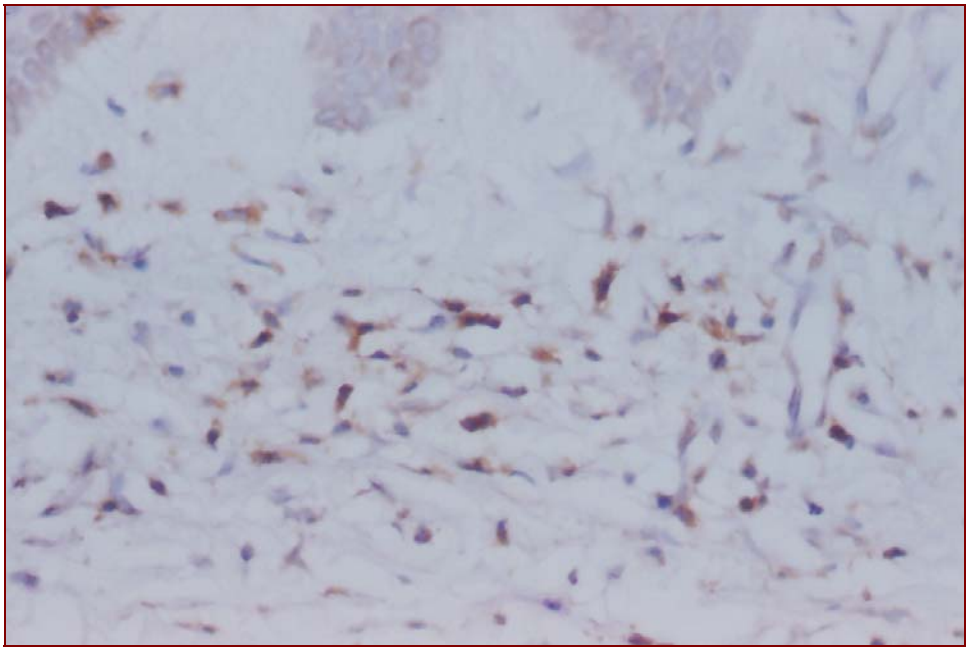


**Figure 17 Photomicrograph of CD45RO expression in Normal Mucosa (40x)**



# *Discussion*

Lichen planus (LP) is a common mucocutaneous disease. It was first described by Erasmus Wilson in 1869 and is thought to affect 0.5- 1% of the world's population. The condition can affect either the skin or the mucosa or both[44]. It often affects the oral mucosa and may occur without skin lesions. About 50% of the patients with skin lesions have oral lesions[28].

LP represents a cell mediated immunological response to an induced antigenic change in skin or mucosa[28]. A wide variety of drugs have been associated with precipitating LP like eruptions and this phenomenon has been termed as Lichenoid Drug Eruption (LDE). Though individual drugs may be involved, multiple systemic drug therapy, topical agents and contact allergens may also be involved, perhaps by a synergistic action on the host immune system and precipitate LDE. These LDE occurring in oral mucosa are termed as Lichenoid Mucositis (LM) [38].

Because LM can resemble LP clinically and histopathologically, it is not always possible to conclude that a drug has induced LP or LM. Furthermore the identification of the offending drug can be complicated by the factors such as simultaneous exposure to several drugs, drug interactions and variability in the latent period between intake of the drug and appearance of the eruptions[23].

The mean age of the patient with LP was reported by **Fellner** and **Halevy** (1993) to be 47 yrs and 49 yrs respectively .The mean age of the patient for LM according to **West *et al*** (1990) & **Halevy** (1993) was 57 & 66 yrs respectively[23]. In our study the mean age of 15 patients with LP was 36(± 17) yrs for males & 44(± 17) yrs for females and for the 15 patients with LM, it was 38(± 17) yrs for males & 40(± 11) yrs for females. There was only a minimal difference between the mean age of patients with LP

and LM when compared with the studies cited above. This shows that LM could occur in people of younger age groups.

**Jungell P** (1991)[28] reported that 60-65 % of patients affected by LP are females. **Kovesi G** and **Banoczy J** (1973)[34] studied 326 patients with LP & reported that 63% had female predominance. **Lamey PJ *et al*** (1995) [38] studied 161 patients of LM out of which 119 were females. In our present study out of 15 cases, 10 were females and 5 were males in both LP & LM. This finding of female predilection was found to be similar with the previous studies. In our study among the groups (LP, LM, NM) we did not observe any significant difference in the age and gender distribution.

In our study the common site of occurrence of LP was buccal mucosa followed by alveolar mucosa & gingiva and this finding is consistent as the site reported by **Jungell P** (1991)[28] & **Mollaoglu N** (2000)[44]. Similarly the common site of LM reported by **Myer SL *et al*** (2002)[45] was buccal mucosa, which is consistent with our study as 9 cases occurred in buccal mucosa and other sites were reported in tongue, alveolar mucosa and gingiva.

**Jungell P** (1999)[28], **Mollaoglu N** (2000)[44], **Chainami Wu** (2001)[9] reported cases of LP in the buccal mucosa showing bilateral distribution. In our study 12 (80%) cases showed bilateral distribution. LP generally has a period of remission and exacerbations; the possibility of different remission periods between the two sides may have been the cause for the unilateral distribution seen in 3 (20 %) of our cases.

**Halevy** (1993)[23] & **McCartan** (1997)[42] stated that clinically LM has a unilateral presentation. In our study, 14 (93%) cases showed unilateral distribution, which is consistent with the previous reports, cited above. However 1 (6.7%) case in our study had multiple amalgam fillings and this case showed bilateral distribution.

LC plays a major role in the pathogenesis of LP as it recognizes, processes and presents the antigen to both helper and cytotoxic T cells specific for protein antigens, haptens, and alloantigens[39]. These dendritic cells initiate immune reactions, and the lesions of LP are characterized by sub and intra-epithelial accumulation of T lymphocytes associated with basal cell destruction[20].

To identify human LC in the epithelium, the most reliable marker used is CD1a (T6) antibody[39]. We have used this antibody to evaluate LC number and activity in LP, LM, NM. We have considered those cells showing membranous and weakly cytoplasmic staining pattern as CD1a positive LC cells.

We observed that the mean CD1a labeling index (LI) for LP was significantly higher than that of LM & NM in the basal and supra basal layer. The mean CD1a positive cells in the connective tissues for LP was higher than that of LM and NM. The difference in mean LI among the study groups showed statistical significance. These observations were consistent with that of **McCartan *et al*** (1997)[41], **Regezi *et al*** (1985)[50], **Laine J *et a*l** (1999)[37], who reported statistically significant higher number of LC in LP. However they did not observe significant difference of CD1a LI between the different layers of the epithelium of LP.

**Eversole *et al*** (1994)[17] and **Farthing PM *et al*** (1990)[20] observed dendritic cells in both the epithelium and the underlying connective tissue, involved in antigen processing and presentation to CD4 helper lymphocytes in LP.

LC in OLP has been shown to be either increased in number or activated indicating the preparation for boosted antigen presentation[37]. Absolute numbers of LC in normal and diseased tissues are difficult to establish because the number of LC vary from one oral focus to another and from one patient to another[50]. Not all the results are comparable as a variety of staining techniques and counting techniques have been employed: including counting per mm of basal layer, counting per mm of epithelial surface, per $mm^2$ of epithelium and per high power field direct counts and counts from photomicrographs [41].

**Regezi *el al*** (1985)[50] postulated that although there was an actual influx of additional LC into the epithelium in active state of LP, it could be argued that this may be more apparent than real. Increased metabolic activity and antigenic challenge could account for greater production of HLA-DR antigen expression in resident LC without real influx of LC. In our study, there was a significant difference in the mean difference CD1a LI between LP and LM in basal, supra basal layers of epithelium and in the connective tissue.

**McCartan *et al*** (1997)[41] concluded that in OLP the initiation of the process appears to be permeation of antigens into the oral epithelium followed by the entrapment of the trans-epithelial antigen by LC. In LM associated with systemic drug administration, the route of access of the antigen to the immune system is unlikely to be directly across the epithelium but probably involves a remote site of antigen processing

and presentation, which would be expected to result in reduced number of LC in the oral epithelium. In our study also our findings indicated decreased number of LC in LP than LM, as established by mean CD1a LI. The reduced LC count in LM as compared with LP argues for two different routes of antigen presentation and processing[41].

In our study we evaluated the mean difference in mean CD1a LI between LM and NM. The mean difference was higher in LM than NM and this difference was significant only in suprabasal layer than in basal or connective tissue layer. This observation can be explained as stated by **McCartan *et al*** (1997)[41] that when LM occurred in response to topical agents and contact allergens, the route of antigen would probably be across the epithelium in which case the number of activated LC might be expected to equal those found in LP[41].

**Laine J *et al*** (1999)[37] reported that MHC class II expression by LC reflects a different route of antigen penetration (exogenic Vs endogenic). Reduced activity of LC has been shown in LM as compared to OLP.

**Dorrego MV *et al*** (2002) [16] concluded that in LP the immunological reaction begin with LC activation, which presents the antigen to CD4 lymphocytes. These cells through ICAM 1 and VCAM 1 expression can activate CD8 lymphocyte leading to a chronic form of the disease.

**Sloberg *et al*** (1984)[58] considered that the increase in expression of LC in LP is a property of LC to improve their capacity to detect and present the antigen to the subepithelial T lymphocytes. The increased expression of Ia-like antigens on LC and the contemporary finding of Ia-like antigens on the subepithelial T-cells, support the opinion that the pathogenesis of OLP is mainly a cell-mediated type of immunological reaction.

In our study, mild CD1a expression was seen in the basal layer of 53.3 % LP as compared to LM, which was 60%, and in NM it was 80%. Intense staining was found only in 4% of LP. In the supra basal layer CD1a expression was mild in 26.7% and moderate in 46.7% of LP when compared with LM which was 66.7% and 2% respectively. The expression in NM was 40% in both mild and moderate intensities. However 6.7% intense staining was found only in LP. Though we observed a difference in the staining intensity pattern between the study groups, the difference was not significant. Similarly, CD1a expression in connective tissue of LP and LM shows 33.3% as mild & 46.7% as moderate staining while NM showing only 60% mild staining. However 20% intense staining was observed only in LP compared to LM & NM and this was statistically significant.

Our observations, in which there is a significant increase in the expression of CD1a in different layers of epithelium and in connective tissue when compared to LM suggests the active role of LC in the pathogenesis of LP and the possible role of presentation of antigen to the T lymphocytes.

**Mega H *et al*** (2001)[43], **Porter *et al*** (1997)[49] and **Farthing PM *et al*** (1990)[20] reported a significant increase in expression of LC in LP than in NM. There is a significant increase in HLA-DP, HLA-DQ and HLA-DR expression in LP possibly induced by local cytokines production.

**Laine J *et al*** (1999)[37] reported 19 cases of amalgam associated LM, in which 5 cases had a higher expression of LC in the epithelium than in NM. They attributed this reaction to the trans-epithelial route of entrance of metal haptens released from dental restorative materials. The results obtained from various studies were consistent with our

present study. This suggests that in LP, LC is immunologically active and plays a role in lesion development.

LP is an interface reaction consisting typically of T lymphocytes infiltration in the upper layer of connective tissue in close apposition to the basal layer of epithelium.[41] Both CD4 and CD8 T Cells are found in LP. The majority of the lymphocytes in the infiltrate are CD8 and CD45RO memory positive cell. The latter cell sub type is not normally found in healthy mucosa[21]. CD4 T cells, which express CD45RO antigen, are called memory cells and they proliferate in response to recall antigen. These cells show stronger helper function for the production of antibody. Naive T cell loses the CD45RA antigen after activation and begins to express CD45RO[69].

In our study memory T cells were identified by using CD45RO antibody. The present study demonstrated a higher expression of CD45RO in connective tissue layer of LP (53.3% intense staining) when compared to LM (20% intense staining) and no intense staining in NM. However NM showed 60% mild & 30% moderate staining. This difference was statistically significant. In LP, there are two populations of CD4 helper cell. One is CD45RA with suppressor inducer activity and the second is CD45RO, which serves as memory cell. The latter group predominates in LP[5].

**Porter SR *et al*** (1997) [49] reported that in LP majority of T lymphocytes express αβ TCR and are CD45RO +ve memory T cells. **Walton LJ *et al*** (1998) [66] reported that circulating memory CD45RO T cells were increased from 49.1% in controls to 65.5 % in LP.

**Rudrigues Nunez *et al*** (2001)[52] reported the mean proportion of CD45RO lymphocyte with higher expression in atrophic erosive LP than in patients with reticular LP. **Kirby C *et al*** (1995)[32] reported the presence of lymphocytes function associated antigen 3 (LFA-3) on LC. This may be an important aspect of disease development since in OLP, LC are highly activated. These cells also express VCAM-1 and ICAM-1 and present the antigen to memory T- cells (CD45RO), the predominant phenotype in LP.

**McCartan *et al*** (1997)[42] reported that although the T cell numbers are lower in LM than LP, the CD4/CD8 ratio is similar in these two conditions. Lymphocyte in LM was that of T helper lymphocyte with a suppressor/inducer phenotype CD45RA+ve/CD29-ve predominance in connective tissues infiltrate, while in LP the cell type is T helper/inducer cell expressing activation and proliferation antigen CD45RA -ve / CD29 +ve / HLADP +ve[41] .

In LP lymphocytes are recruited into the mucosa by upregulation of adhesion molecule expression, possibly driven by cytokines. The damage to keratinocytes and the basement membrane is predominantly cytotoxic T lymphocytes mediated. Furthermore the activation of T lymphocytes and the resultant generation of cytokines are likely to perpetuate that LP is a cell-mediated immune response[49].

In LP the inflammatory infiltrate consists of significant number of CD4 T cells in the lamina propria and CD8 T cells in close proximity to epithelial basement membrane. Accumulation of CD8 cells seems to increase gradually in disease progression[15]. CD4 helper T cells are stimulated to secrete Th1 cytokine IL2 & IFNγ. Subsequently CD8 cytotoxic T cells may be activated by the combination of antigen associated with MHC Class I on basal keratinocyte and by CD4 T cell derived IL2 & IFN γ. Activated CD8

cytotoxic T cell trigger basal keratinocytes apoptosis in LP[31]. In our study also the CD45RO staining intensity in LP was higher than in LM & NM. These results were consistent with the previous studies cited above.

LP is triggered by some events that cause CD4/CD45RA +ve lymphocytes to differentiate into CD4/CD29+ve lymphocytes. From this point, immunosuppressive mechanisms are reduced, leading to an accumulation of memory CD45RO T cell[52].

The results of the present study demonstrate that there is a significant increase in the number and expression of LC and expression of memory T helper cell in LP than in LM. This clearly indicates that the route of antigen presentation and penetration is different between these two lesions. However in LM due to topical agents or contact allergens, it is possible that the route of antigen presentation could be trans-epithelial, which is different from the LM associated with systemic drug administration.

# *Summary & Conclusion*

- A total of 40 patients were included in our study, comprising of 15 patients of LP, 15 LM and 10 NM.
- In our study, the mean age of LP was 36(± 17) years for males and 44(± 12) years for females, whereas LM was 38(± 17) years for males and 40(± 11) years for females. Both LP & LM showed a female predominance.
- Buccal mucosa was the affected site in all cases of LP, however 60% cases of LM also showed the same site of occurrence. 80% LP showed bilateral distribution whereas 93% of LM had unilateral distribution.
- Immunohistochemical study was done to evaluate the number and expression of LC, and to demonstrate the expression of T cells in LP, LM & NM.
- Increased number of LC was observed in LP, in the basal and supra basal layer of epithelium and in the connective tissue compared to LM and NM by using CD1a staining.
- Increased intensity of expression of LC was observed in LP, in basal and supra basal layer of epithelium compared to LM, NM and this was not statistically significant.
- Increased CD1a expression was observed in the connective tissue in the sequence of LP> LM > NM and this was statistically significant.

- Increased CD45RO expression was observed in the connective tissue in the sequence of LP> LM > NM and this difference in staining intensity was statistically significant.
- The present study clearly demonstrates a statistically significant increase in number and expression of LC and also an increase in the expression of memory T cell in LP than in LM, indicating the possible different immunopathogenic mechanisms.

# *Bibliography*

1. **Abbas AK and Lichtman AH.**

   Cellular and Molecular immunology, 5th edition.

   *WB Saunders Company publication, 2000; 110-115.*

2. **Arachchi PA, Crane IJ, Scully C.**

   Epithelial dendritic cells in pathological human oral tissues.

   *J Oral Pathol Med 1989; 18: 11-6.*

3. **Bhan AK, Harrist TJ, Murphy GF, Mihm MC.**

   T cell subsets and Langerhans cells in lichen planus: in situ characterization using monoclonal antibodies.

   *Br J Dermatol1981; 105(6): 617-22.*

4. **Bolewska J and Reibel J.**

   T lymphocytes, Langerhans cells and HLA-DR expression on keratinocytes in oral lesions associated with amalgam restorations.

   *J Oral Pathol Med. 1989; 18: 525-8.*

5. **Boyd AS.**

   Update on the diagnosis of lichenoid dermatitis.

   *Adv. Dermatol 1996; 11: 287-307.*

6. **Bramanti TE, Dekker NP, Nur FL.**

   Heat shock proteins and γδ T lymphocytes in oral lichen planus.

   *Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 80: 698-704.*

7. **Campisi G, Giovannelli L, Arico P.**

HPV DNA in clinically different variants of oral leukoplakia and lichen planus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98: 171-83.*

8. **Chainani-Wu N, Lozada-Nur F, Terrault N.**

Hepatitis C virus and lichen planus a review.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod.2004; 98: 17-83.*

9. **Chainani wu N, Silverman S JR, Lozada-Nur F, Mayer P, Watson JJ.**

Oral lichen planus: patient profile, disease progression and treatment responses.

*JADA 2001; 132: 901- 9.*

10. **Chaiyarit P, Kafrawy AH, Miles DA.**

Oral lichen planus – an immunohistochemical study of heat shock proteins and cytokeratins & a unifying hypothesis of pathogenesis.

*J Oral Pathol Med 1999; 28: 210-5.*

11. **Charbit Y, Monteil RA, Hitzig C.**

S100 immunolabelling of LC in oral epithelium.

*Oral Pathol 1986; 15: 419-22.*

12. **Chaudhary S.**

Psychological stressors in oral lichen planus.

*Aust Dent J 2004; 49: 192-5.*

13. **Chou MJ and Daniels TE.**

Langerhans cells expressing HLA-DQ, HLA-DR and T6 antigens in normal oral mucosa and lichen planus.

*J Oral Pathol Med 1989; 18(10): 573-6.*

14. **Cunha KS, Manso AC, Cardoso AS.**

Prevalence of oral lichen planus in Brazilian patients with HCV infection.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 330-3.*

15. **Derossi SS and Ciaerocca KN.**

Lichen planus, lichenoid drug reactions, and lichenoid mucositis.

*The Dental Clinics of North America 2005; 49: 77-89.*

16. **Dorrego VM, Correnti M, Delgado R, Tapia FG.**

Oral lichen planus: immunohistology of mucosal lesions.

*J Oral Pathol Med 2002; 31: 410-4.*

17. **Eversole LR.**

Immunopathology of oral mucosal ulcerative, desquamative and bullous diseases.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1994; 77: 555-71.*

18. **Fan J, Schoenfeld RJ, Hunter R.**

A study of the epidermal clear cells with special reference to their relationship to the cells of Langerhans.

*J invest Dermat 1958; 21: 445-450.*

19. **Farthing PM, Matear P, Cruchley AT**.

Langerhans cell distribution and keratinocyte expression of HLADR in oral lichen planus.

*J Oral Pathol Med 1992; 21: 451-5.*

20. **Farthing PM, Matear P, Cruchley AT.**

The activation of Langerhans cells in oral lichen planus.

*J Oral Pathol Med 1990; 19: 81-5.*

21. **Fitzpatrick.**

Dermatology in General Medicine 5th Edition, Vol 1.

*Mc Graw – Hill Publication, 1999; 463-477.*

22. **Garcia-Pola MJ, Huertaa G, Cereros R.**

Anxiety and depression as risk factors for oral lichen planus.

*Dermatology 2001; 203: 303-307.*

23. **Halevy S and Shai A.**

Lichenoid drug eruptions.

*J Am Acad Dermatol 1993; 29: 249-55.*

24. **Hasseus B, Jontell M, Brune M, Johansson P, Dahlgren UI.**

Langerhans cells and T cells in oral graft versus host disease and oral lichen planus.

*Scand J Immunol 2001; 54: 516-24.*

25. **Issa Y, Brunton PA, Glenny AM.**

Healing of oral lichenoid lesions after replacing amalgam restorations:A systematic Review.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004; 98: 553-65.*

26. **Issa Y, Duxbury AJ, Macfarlane TV.**

Oral lichenoid lesions related to dental restorative materials.

*Br Dent J 2005; 26: 361-6.*

27. **Jakob Tand Udey MC.**

Epidermal Langerhans cell – From Neurons to Nature's adjuvant.

*Adv. Dermatol 1999; 14: 209-259.*

28. **Jungell P.**

Oral Lichen planus review.

*Int J Oral Maxillofac Surg 1991; 20: 129-31.*

29. **Katou F, Ohtani H, Saaristo A.**

Immunological activation of dermal Langerhans cell in contact with lymphocytes in a model of human inflamed skin.

*Am J Pathol 2000; 156: 519-527.*

30. **Kawamaura E, Nakamura S, Sasaki M.**

Accumulation of oligoclonal T cells in the infiltrating lymphocytes in Oral lichen planus.

*J Oral Pathol Med 2003; 32: 282-9.*

31. **Khan A, Farah CS, Savage NW.**

Th 1 cytokines in oral lichen planus.

*J Oral Pathol Med 2003; 32: 77-83.*

32. **Kirby AC, Olsen I, Farthing PM, Porter SR.**

Expression of lymphocyte function-associated antigen 3 in oral lichen planus. *Oral Disease 1995; 1: 193-7.*

33. **Koray M, Dugler O, Horasanli S**

Evaluation of anxiety and salivary cortisol levels in patients with oral lichen planus.

*Oral Disease 2003; 9: 298-05.*

34. **Kovesi G and Banoczy J.**

Follow-up studies in oral lichen planus.

*Int J Oral Surg 1973; 2: 13-9.*

35. **Lacy MF, Reade PC, Hay KD.**

Lichen planus – A Theory of Pathogenesis.

*J Oral Surg 1983; 56: 521-5.*

36. **Laeijendecker and Van J.**

Oral manifestations of gold allergy.

*J Amer Acad Derm1994; 30: 205-9.*

37. **Laine J, Happonen RP, Vainio O.**

Immunocompetent cells in amalgam associated oral lichenoid contact lesions.

*J Oral Pathol Med 1999; 28: 117-121*.

38. **Lamey PJ, McCartan BE, MacDonald.**

Basal cell cytoplasmic autoantibodies in oral lichenoid reactions.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 79: 44-9.*

39. **Lombardi T, Hauser C, Budtz-Jorgensen E.**

Langerhans cells: structure, function and role in oral pathological conditions.

*J Oral Pathol Med 1993; 22: 193-202.*

40. **McCartan BE.**

Psychological factors associated with oral lichen planus.

*J Oral Pathol Med 1995; 24: 273-5.*

41. **McCartan BE and Lamey PJ.**

Expression of CD1 and HLA-DR by Langerhans cells in oral lichenoid drug eruptions (LDE) and idiopathic oral lichen planus.

*J Oral Pathol Med 1997; 26: 176-80.*

42. **McCartan BE and McCreary CE.**

Oral lichenoid eruptions.

*Oral Diseases 1997; 3: 58-63.*

43. **Mega H, Jiang WW, Takagi M.**

Immunohistochemical study of oral lichen planus associated with hepatitis C virus infection, oral lichenoid contact sensitivity reaction and idiopathic oral lichen planus.

*Oral Diseases 2001; 7: 296-305.*

44. **Mollaoglu N.**

Oral lichen planus.

*British Journal of Oral & Maxillofacial surgery 2000; 38: 370-77.*

45. **Myers SL, Rhodus NL, Parsons HM, Hodges JS, Kaimal S.**

A retrospective survey of oral lichenoid lesions: revisiting the diagnostic process for oral lichen planus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93: 676-81.*

46. **Penneys NS, Ackerman B, Gottlieb.**

Gold dermatitis – a clinical and histopathological study.

*Arch dermatol, 1974; 109: 372-376.*

47. **Pinkus H.**

Lichenoid tissue reactions.

*Arch Dermatol 1973; 107: 840-6.*

48. **Porter K, Klouda P, Scully C.**

Class I and II HLA antigens in british patients with oral lichen planus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1993; 75: 176-80.*

49. **Porter SR, Kirby A, Olsen I.**

Oral and maxillofacial pathology immunologic aspects of dermal and oral lichen planus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 8: 358-66.*

50. **Regezi JA, Stewart JC, Lloyd RV, Headington JT.**

Immunohistochemical staining of Langerhans cells and macrophages in oral lichen planus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1985; 60: 396-402.*

51. **Reichart A and Grote.**

The man behind the eponym – Paul Langerhans.

*J Oral Pathol Med 2001; 30: 255-6.*

52. **Rodriguez-Nunez I, Blanco-Carrion A, Garcia AG, Rey JG.**

Peripheral T-cell subsets in patients with reticular and atrophic-erosive oral lichen planus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91: 180-8.*

53. **Sand LP, Jalouli J, Larsson PA.**

Prevalance of EBV in oral squamous cell carcinoma, oral lichen planus and normal mucosa.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93:586-92.*

54. **Santoro A, Majorana A, Roversi L, Gentili F.**

Recruitment of dendritic cells in oral lichen planus.

*J Pathol 2005; 205: 426-34.*

55. **Sato M, Tokuda N, Fukumoto T.**

Immunohistopathological study of the oral lichenoid lesions of chronic GVHD.

*J Oral Pathol Med 2006; 35: 33-6.*

56. **Scully C and Diz DP.**

Orofacial effects of antiretroviral therapies.

*Oral Diseases 2001; 7: 205-10.*

57. **Segura-Egea JJ, Bullon-Fernandez P.**

Lichenoid reaction associated to amalgam restoration.

*Med Oral Pathol Oral Cir Bucal 2004; 9: 423-4.*

58. **Sloberg K, Jonsson R, Jontell M.**

Assessment of Langerhans' cells in oral lichen planus using monoclonal antibodies.

*J Oral Pathol 1984; 13: 516-24.*

59. **Sugerman PB, Savage NW, Walsh LJ.**

The pathogenesis of oral lichen planus.

*Crit Rev Oral Biol Med 2002; 13: 350-365.*

60. **Sugerman PB, Savage NW, Walsh LJ.**

Disease mechanism in oral lichen planus, a possible role for autoimmunity. *Australas J Dermatol 1993; 34: 63-9.*

61. **Takeuchi Y, Tohani I, Kaneda T.**

Immunohistochemical analysis of cells in mucosal lesions of oral lichen planus.

*J Oral Pathol 1998; 17: 367-373.*

62. **Van Dis ML and Parks ET.**

Prevalence of oral lichen planus in patients with diabetes mellitus.

*Oral Surg Oral Med Oral Pathol Oral Radiol Endodont 1995; 79: 696-700.*

63. **Villarroel DM, Correnti M, Delgado R, Tapia FJ.**

Oral lichen planus- Immunohistology of mucosal lesions.

*J Oral Pathol Med 2002; 31: 410-4.*

64. **Walsh LJ.**

Mast cells and oral inflammation.

*Crit Rev Oral Biol Med 2003;14: 188-98.*

65. **Walsh LJ, Tseng PW, Savage NW, Seymour GJ.**

Expression of CDw29 and CD45R antigens on epithelial cells in oral lichen planus.

*J Oral Pathol Med 1989; 18: 360-5.*

66. **Walton LJ, Macey MG, Thornhill MH, Farthing PM.**

Intra-epithelial subpopulations of T lymphocytes and Langerhans cells in oral lichen planus.

*J Oral Pathol Med 1998; 27:116-23.*

67. **Walton LJ, Thornhill MH, Farthing PM.**

VCAM-1 and ICAM-1 are expressed by Langerhans cells, macrophages and endothelial cells in oral lichen planus.

*J Oral Pathol Med 1994; 23: 262-8.*

68. **Winning T, Gemmel E, Polak B.**

Expression of CD1a on monocytes cultured with supernatants from peridontally diseased gingival epithelial cells.

*Oral Diseases 1996; 2: 247-252.*

69. **Yamazaki K, Nakajima T, Aoyagi T.**

Immunohistological analysis of memory T lymphocytes and activated B-lymphocytes in tissues with periodontal disease.

*J Periodontal Res 1993; 28: 324-34.*

70. **Zhao ZZ, Savage NW, Sugerman PB.**

Mast cell/ T cell interaction in oral lichen planus.

*J Oral Pathol Med 2002; 31: 189-95.*

71. **Zhao ZZ, Sugerman PB, Walsh LJ.**

Expression of RANTES abndCCR1 in oral lichen planus and association with mast cell migration.

*J Oral Pathol Med 2002; 32: 158-62.*

72. **Zhao ZZ, Sugerman PB, Zhou XJ.**

Mast cell degranulation and the role of T cell RANTES in oral lichen planus.

*Oral Diseases 2001; 7: 246-51.*

73. **Zhou XJ, Sugerman PB, Savage NW.**

Intra epithelial CD8 T cells and basement membrane disruption in oral lichen planus.

*J Oral Pathol Med 2002; 31: 23-7.*

# *Annexure*

LICHENOID MUCOSITIS INDUCING DURGS[23]

- **Antimalarials**

    Quinacrine and Chloroquine

    Quinine and Quinidine

- **Antihypertensive agents**

    β-adrenergic blocking agents

    Angiotensin-converting enzyme inhibitos

    Methyl dopa

- **Diuretics**

    Thiazide diuretics

    Furosemide

    Spironolactone

- Sulfonyl urea hypoglycemic agents
- Non-steroidal anti-inflammatory drugs
- Gold salts
- Pencillamine
- Tetracycline
- Allopurinol
- Ketaconozole
- **Heavy metals**

    Mercurials

    Arsenicals

    Bismuth